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BIOLOGICAL CONTROL OF WEEDS  
LABORATORY-EUROPE  
Rome, Italy

1983 ANNUAL REPORT



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BIOLOGICAL CONTROL OF WEEDS LAB PERSONNEL

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# TABLE OF CONTENTS

Introduction	page	4
Leafy Spurge	page	5
<u>Chamaesphecia</u> sp., <u>Hyles euphorbiae</u> , <u>Oberea erythrocephala</u> , <u>Dicranocephalus</u> spp. (Rizza)		
<u>Dasineura capsulae</u> (Pecora)	page	12
<u>Aphthona abdominalis</u> (Pecora)	page	21
<u>Bayeria capitigena</u> (Pecora)	page	24
<u>Euphorbia</u> spp. (Sobhian)	page	27
<u>Centaurea solstitialis</u>	page	31
<u>Urophora siruna-seva</u> (Sobhian)		
<u>Bangasternus</u> spp. (Sobhian)		
<u>Chaetorellia hexachaeta</u> (Sobhian)	page	40
<u>Centaurea solstitialis</u>	page	47
<u>Apion</u> spp. (Clement, Cristofaro, Mimmocchi)		
<u>Centaurea solstitialis</u>	page	61
<u>Bangasternus orientalis</u> (Dunn, Campobasso, Murano)		
<u>Cyphocleonus morbillosus</u> (Dunn, Campobasso)	page	69
<u>Eustenopus villosus</u> (Sobhian)	page	76
<u>Centaurea diffusa</u>	page	84
<u>Pterolonche inspersa</u> (Sobhian)	page	85
<u>Bangasternus provincialis</u> (Sobhian)	page	86
Gall Wasp (Sobhian)	page	87
<u>Larinus minutus</u> (Sobhian)		
<u>Pterolonche inspersa</u> (Dunn, Campobasso)	page	88
<u>Sphenoptera jugoslavica</u> (Campobasso)	page	89
<u>Abutilon theophrasti</u>	page	90
<u>Carcharodus</u> sp. (Sobhian)		
<u>Galium</u> spp.	page	94
<u>Eriophyes</u> sp., <u>Dasineura</u> sp. (Clement, Cristofaro)		
<u>Rumex</u> spp.	page	104
<u>Pyropteron chrysidiforme</u> (Clement, Cristofaro)		
Publications	page	106
Travels	page	107
Insect shipments	page	110
Visitors	page	111



## INTRODUCTION

The year 1983 was an eventful year for the Rome Laboratory. Some of the events were positive, and we hope some are never repeated.

On October 20 a terrorist group left a bomb at the laboratory, severely damaging the building. Fortunately the bombing occurred when the building was unoccupied so no one was killed or injured by the blast. The work of repairing the building went on into 1984.

One of the positive events was the first in-depth review of the Rome Laboratory by a Blue Ribbon review team of USDA and extramural scientists and cooperators from both the United States and Europe. The recommendations of the review panel had both immediate and long range influence on the laboratory's program. The laboratory staff was pleased to have participated in the review, and as a result we have a more cohesive program.

Among the recommendations of the review team was a very important one, recommending purchase of the present laboratory, if zoning variance can be obtained. In 1983 we found that the laboratory has a clear title and started to work on the zoning variance problem.

Most of our scientific objectives were completed, and from this point of view it was a productive year.

Also, during the year, Dott. Pasquale Pecora was given the full responsibility of a Research Entomologist and Dott.ssa Tiziana Mimmocchi was awarded a scholarship from the University of Melbourne and went there for a 1 year study in plant pathology.

Paul H. Dunn  
Research and Location Leader



## LEAFY SPURGE

Antonio Rizza, Lead Scientist

(prepared by Paul Dunn)

Leafy spurge is a complex of species including Euphorbia esula and virgata. It was accidentally introduced into North America from Europe and has become a serious weed problem in the northern tier of states and five Canadian provinces. In the US the states with the worst problems are Minnesota (800,000 acres), North Dakota (600,000 acres) Montana (543,000 acres). Several other states have lesser but serious infestations. Despite heavy expenditures for control this weed is spreading at an alarming rate, currently infesting about 2,500,000 acres. A conservative estimate of the cost to US Agriculture caused by this weed, in terms of expenditure for control and loss of productivity is about \$10.5 million annually.

In May 1983 the leafy spurge program at the Rome Laboratory was reviewed by Dr. Lloyd Andres, acting as Technical Advisor. He recommended that the emphasis for the spurge program at the Rome laboratory for 1983 through 1986 should be placed on collecting, cleaning up and shipping climatically adapted species that have already been cleared for release in the United States.

It was also recommended that a culture of the Coreid bugs Dicranocephalus spp. and the eriophyid mite Eriophyes euphorbiae be collected, colonies started at the Rome Laboratory, and that observations on the large root feeding weevil Neoplinthus tigratus continue.

The spurge program was effectively divided into two parts, the collection phase and the development of new candidates phase.

Mr. Rizza was in charge of the collections and the work with Dicranocephalus and Eriophyes while Dott. Pecora was charged with developing other new natural enemies. Collections and shipments were made of the following species:

Aphthona flava Guill., Aphthona cyparissiae Koch, Chamaesphecia prob. tenthrediniformis, Hyles euphorbiae, Oberea erythrocephala. In addition roots of the Euphorbia esula-virgata group were collected in eastern Europe and sent to Canada for taxonomic studies.

The flea beetles, Aphthona flava and A. cyparissiae were both requested by Dr. Robert Pemberton at Albany so he was sent 300 A. flava collected in June at San Rossore (Pisa) and 100 A. cyparissiae collected on Euphorbia esula (sensu strictu) and another 100 collected on Euphorbia cyparissias in Austria and Hungary. In addition 1300 Aphthona flava (from the same San Rossore stock as the Pemberton material) were sent to Dr. Peter Harris at Regina, Saskatchewan at the request of Dr. Dieter Shroeder, C.I.B.C., Delemont.

Chamaesphecia sp.

In November 1982 550 roots of Euphorbia esula (sensu latu) plants infested with Chamaesphecia sp. larvae were collected in Hungary. These roots were brought to Rome to overwinter. On April 19, 1983 500 of these infested roots were sent to the quarantine at Albany to be emerged and released. The other 50 roots were retained at Rome where the adults were emerged and allowed to oviposit. Viable eggs from these adults were transferred to North American leafy spurge varieties from Montana, Nebraska, Nevada, North Dakota, Oregon, and Utah. Unfortunately, larval survival occurred only on the control plants (E. esula sensu latu) on which the insect was found.

Hyles euphorbiae

Since the Hyles moths that were first introduced into North America were all collected from Euphorbia cyparissias in Europe it seemed logical to collect a biotype from E. virgata which may be better adapted to the American leafy spurge. In June, over 150 larvae of Hyles were collected from E. virgata in Hungary and reared to the pupal stage during the trip. From these pupae brought to the Rome laboratory a second generation emerged in July and 1,500 fertile eggs of this E. virgata strain were sent to Albany for release. In August, 85 pupae of the same stock were also sent to Albany so they would have additional material to release in 1984.

Oberea erythrocephala

In June and July, 240 O. erythrocephala adults were collected at San Rossore (Pisa). One hundred of these were sent to Dr. Delucchi at Zurich for use in artificial diet studies being made under a USDA contract. The other 140 insects were sent to Albany for field release.

In addition, another 50 O. erythrocephala adults were collected from Euphorbia virgata in Hungary, and these were also sent to Albany for release. These insects were not abundant but by hand picking and sweeping 6-7 per day could be found. At any rate, these insects from E. virgata should be better adapted to the US leafy spurge therefore accept them more readily than the Oberea collected from Euphorbia esula and Euphorbia cyparissias. In June 1984 we expect to make another trip to Hungary to collect more Oberea on E. virgata in order to establish a colony in Rome and mass rear the insect in large cages on US leafy spurge varieties in order to have a larger number of insects to send in 1985. These insects will have already passed one generation on U.S. spurge which may aid in their establishment, once released.

Dicranocephalus spp.

Two experiments were made with Dicranocephalus spp. The first was to determine if the insect would accept American leafy spurge plants as hosts and the second trial was to determine the number of nymphal instars.

Experiment 1. Acceptance of American Spurges

Using eggs that were layed by adult Dicranocephalus sp. collected in Hungary as an infestation source 5 1st instar nymphs were placed on each of 10 leafy spurge test plants, four on one test plant and 6 on another two for a total of 71 nymphs. Ten of the plants were from 6 different states in the United States, ie. Iowa, Oregon, Michigan, Minnesota, Montana and North Dakota, one plant was from Russia, one from Pisa, Italy and the other was a different species (E. characias) from Italy. There was no control plant of Hungarian origin.

Results

One female arrived to the adult stage on an Oregon plant and a male matured to the adult stage on a Montana plant. The female required 40 days until the final molt and the male 32 days. Two nymphs lived 34 days on one Oregon plant and two other nymphs lived 16 and 23 days on another Oregon plant. None of the other nymphs lived long enough to complete the molt to second instar. Since there was no control, these results are largely inconclusive. It is not clear if the high pre-imago mortality (97%) was due to faulty rearing procedures or non acceptance of the leafy spurge plants offered. The only conclusion that can be drawn from this trial is that a small percentage of Dicranocephalus sp. found on leafy spurge in Hungary can complete development on U.S. varieties of leafy spurge. The trial should be repeated with a control plant from Hungary.

## Experiment 2. Nymphal Stages of *Dicranocephalus* sp.

One first instar nymph was placed on each of 7 bouquets of its host plant, *Euphorbia esula* from Pisa. Each bouquet had 5 to 6 immature seeds and was housed in a plastic cylinder cage 5 cm Ø and 8 cm high. The bottom of the cage had a hole to which a vial of water was fixed and the cut stems of the bouquets were held in the water by a cotton plug.

### Results

Of the 7 nymphs in the trial 4 matured to the imago stage, passing through 5 molts and requiring 30, 30, 31 and 32 days to complete their development. Several additional observations were also made on these two colonies of *Dicranocephalus*.

1. The insects from Hungary layed 71 eggs. These were probably all produced by 1 female because the other female continued mating after the eggs were found in the cage.

2. The adults were seen to feed on seeds of leafy spurge. They hold the seeds still with their front legs and insert their rostrum to feed.

3. The nymphs do not need seeds to feed on for maturation. Two insects developed from nymphs to adults when supplied bouquets with leaves only, and the resulting adults lived for 40 days on the vegetative material.

### *Neoplinthus tigratus* Observations

On November 15, 1982 15 roots of *E. esula* infested with mature larvae of *Neoplinthus tigratus* were collected at San Rossore, Pisa, brought to the Rome Laboratory and replanted in a 38 cm Ø pot and covered with a nylon organdy tube cage and kept in the garden for adult emergence.

An inspection on June 5, 1983 disclosed that 1 adult had emerged. On June 6 all the 5 adults 1♀ and 4 ♂ had emerged. All the five insects were collected and put in a 1 pint ice cream carton with 1 cm moist peat moss on

the bottom and a bouquet of Euphorbia esula (ex Pisa) leaves. The bouquet was checked for feeding injury on 7 July and it was noted that a section of stem about 1.5 cm long had been scraped and 20 mm<sup>2</sup> of leaf had been consumed. The 5 adults were then caged with a leafy spurge plant (E. esula ex Pisa) in a pot. Some moist peat moss (2 cm) was put on the soil of the pot to allow the insects a chance to hide. The plant in the pot was covered with a transparent plastic cylinder cage with a screen top to allow observation of insect feeding damage to the plant.

July 13 - A scraping sort of feeding damage totaling 160 mm<sup>2</sup> was seen on four stems.

August 10 - The pot was checked again and only 3 living and 1 dead adult were seen, as well as 580 mm<sup>2</sup> scraping type feeding damage on 6 plant stems.

September 12 - The pot was checked again. Two living adults were seen and there was no evidence of oviposition. Feeding damage was not recorded.

On the subsequent observation, all the Neoplinthus in the pot were dead so they were collected and pinned.

A new group of 7 Neoplinthus adults were brought to the laboratory by Dott. Enzo Colonnelli in early October. On October 17 the plant on which they had been caged was checked for eggs. There was no sign of oviposition, and the plant was in good condition. These insects were checked again on October 25. Five adults were active (1 pair copulating) but there were no eggs even though the plant was in good condition.

When checking the Colonnelli adults on November 9, 7 were seen; 3 under a small stone, 2 in cracks in the soil, two on the plant and one feeding on a dry leaf. A month later, December 15, seven adults were also found, 3

under pebbles, 2 in a crack in the soil, 2 on the surface (of the soil) and 1 feeding on a dry leaf.

January 18, 1984 the adults were checked and 5 were found alive but inactive, and 2 dead. The plant had senesced but was putting up new shoots.

On the February check, the insects were all dead.

### Conclusions

From these scarce observations it is difficult to conclude much. It was established that the insects feed on the stems and leaves of leafy spurge plants and that the adults will copulate in captivity. Much more work remains to be done in order to understand the biology of this insect enough to manipulate it for host specificity tests.



DASINEURA CAPSULAE

Pasquale Pecora

Dasineura capsulae (Kieffer) is a flower bud gall maker. The gall is formed by the enlargement and deformation of the cyathium; thus seed production is reduced. According to literature records this midge is associated with E. cyparissias L., E. esula L., E. falcata L., E. lucida Waldst and Kit. and E. virgata Waldst and Kit. (Buhr, 1964).

Material and Methods

Biology

To provide data on the adult emergence of D. capsulae, 100 bud galls containing larvae of various stages were collected on E. esula at S. Rossore (Pisa), Italy, on June 15, 1982. These galls were brought to the laboratory and stored in a refrigerator in a closed polyethylene bag (temp. 4°-6°C) for a period of 4-5 days. The low temperature and high humidity, allowed to the mature larvae of D. capsulae to leave the galls. Five days later, using a fine brush 200 mature larvae of D. capsulae, were transferred into a plastic box (L = 24 cm; W = 24 cm; H = 8 cm) with a layer (2 cm) of moistened peat moss on the bottom to provide a suitable substrate for the diapausing insects. Each box was covered by a plastic lid which had a 2 cm diameter central hole plugged with cotton to allow some air exchange. Four containers were prepared and held in a laboratory room, at ambient temperature ranging between 8°C and 25°C.

At the end of March 1983, two of these containers were inspected, and the number of both living and dead larvae of D. capsulae, and the parasitoids associated with them were recorded. The other two containers were left undisturbed until adult emergence started, then the container was checked

daily. Newly emerged adults were transferred, by a mouth aspirator, into acrylic plastic cages (H = 9 cm; diam. = 4 cm). To improve aeration, a hole 2 cm in diameter and covered by nylon organdy was made in the bottom of these cages and the cages inverted so the lid, which served as bottom, was also punctured with a 1.5 cm diameter hole through which the E. esula flower buds (4-6/cage) were passed, thus allowing the insects to be caged directly on the plant. These cages were supported on the plants by fastening them with masking tape to metal rods inserted in the soil of the potted plant. All these cages and plants were held in a laboratory room where the ambient temperature ranged between 10° and 27°C and the RH between 45% and 70%. The RH inside the cages was not determined. This study started on May 2 and ended when the last adult midge died on June 30, 1983. Twenty four (16♀ 8♂) of the newly emerged midges were used to gather biological data of D. capsulae - e.g. oviposition behavior, adult longevity and gall development time. To conduct these studies 8 cages were prepared with 2♀ 1♂/cage. Once the adults were exposed to the host plant (6-8 flower-buds/cage), they were left undisturbed until they died, after which the cages were removed and the buds which had been exposed to the midges were marked. Fifty-six E. esula flower-buds were offered to the adults of D. capsulae and after cage removal, sixteen of these (2 buds/cage) were selected at random and checked for eggs.

To determine the pre-eclosion period and percentage of egg fertility, a sample of 200 of these eggs were collected and placed in hatching containers and held at ambient temperature in a laboratory room. Another stock of 16 flower-buds (2 bud/cage) were dissected a week later, and the behavior of the newly hatched larvae was observed. The remaining galls were left undisturbed until maturity, about 20 days later. At this time all the galls were dissected, and the number and instar of the living and dead larvae found in each gall was recorded. To determine the egg production per female, another 5

cages with 1<sup>0</sup> 1♂/cage were prepared. Three or four buds were exposed to D. capsulae adults and when females died, the buds were dissected and the number of eggs was recorded.

In order to study the phenology of the development of D. capsulae in the field, periodic inspections were made at S. Rossore on April 2, April 19, May 2, May 26, June 15 and July 10, 1983. Each time, depending on which stage was available, a sample of 100 flower buds or 50 galls of various size or both were randomly collected from 10 plants of E. esula. In the inspections made in April only flower bud samples were collected. In the May and June inspections both flower bud and gall samples were taken while in July only galls were collected. Buds and galls were dissected under a dissecting microscope and eggs, living and dead larvae, and parasitoids present were recorded. Also, to provide biometric data on the development of D. capsulae, eggs, the various instar larvae, and adults were measured for length and width under a stereomicroscope. These data are shown in Table 1. Pupae were not measured because they were not available. The size of mature galls was determined measuring length and width of 25 galls.

In another study the effectiveness of a D. capsulae infestation in reducing flower production was determined on plants of leafy spurge at S. Rossore, where a population of this midge occurs naturally. On May 15, May 26, June 15, samples of 20 plants of E. esula, showing traces of D. capsulae infestation, was collected and brought to the laboratory. For each plant the number of flower-buds, flower and galls present were counted, and the % infestation was calculated.

#### Host Specificity

In order to determine if North American biotypes of leafy spurge are suitable hosts for D. capsulae, preliminary tests were conducted from May 15 to June 30, 1983. Adults used in these experiments came from mature larvae

collected on E. esula at S. Rossore in June 1982.

Oviposition and larval survival tests were conducted on leafy spurge from Montana and Oregon with E. esula, from S. Rossore (Pisa), Italy as a control plant. New adults were caged on the test plants in the acrylic plastic cages previously described. Five replicates were made for each test-plant and in each replicate 4-6 flower-buds were exposed to adults of D. capsulae (3♀ 1♂/cage). After cage removal, when the adults had died, two replicates of each test-plant were checked for eggs and the other three were left undisturbed until the galls reached their maturity, then the number of galls and the number of living and dead larvae/gall were recorded. The test-plants were kept inside the laboratory while the adults of D. capsulae were alive. However, after the adults died and were removed these plants were transferred outside to the laboratory garden. In the laboratory room the temperature ranged between 13° and 25°C and the RH between 45-75%; the outdoor temperature ranged between a minimum of 12° C and a maximum of 28°C with a RH of 50 to 80%.

## Results

### Biology

In the laboratory adult emergence of D. capsulae started on May 2 and ended on May 25, 1983. From two plastic containers held for adult emergence of D. capsulae adults 106♀ and 47♂ (38%) emerged. Female longevity (n = 16) was  $3.50 \pm 0.76$  days while the males (n = 8) lived  $1.86 \pm 0.69$  days. The caged adults were never seen mating but the females started to lay eggs either the same day they emerged or the day after. In the cages the females visited 3-5 buds before selecting one for oviposition. Once they selected a suitable flower bud, they extended their ovipositor, inserting it between the two bracts, which cover the cyathium. The time necessary to lay a group of eggs in a bud ranged between 15 and 25 minutes. Only eight of the sixteen E. esula

buds, dissected to discover the oviposition site contained eggs. The average was  $48.12 \pm 19.26$  eggs per bud.

The freshly laid eggs are white, slightly fusiform in shape, with rounded ends, and a smooth soft chorion. In an experiment set up to determine the fecundity of the midge, they deposited a mean of  $86.00 \pm 40.37$  eggs/female which hatched in 3-5 days and were 89% fertile. The measurements of the eggs are reported in Table 1. A second group of buds ( $n = 16$ ) was dissected to ascertain larval behavior but only 6 were infested with D. capsulae larvae containing a mean of  $30.66 \pm 9.52$  young larvae/bud. Newly hatched larvae were found either in the bracts, on the glands or in the upper part of the cyathium, or inside the cyathum. This observation suggests that the neonate larvae, just after hatching, move into the small cup-shaped cyathium.

Several mature galls ( $n = 7$ ) contained a number of mature larvae (mean  $17.00 \pm 6.9$ /gall), all of which were alive. From the two containers (400 mature D. capsulae larvae), held in the laboratory to study the parasitism, 183 larvae (45.75%) were parasitized by Inostemma sp.<sup>1/</sup> (Hymenoptera: Platygasteridae). In addition 25 pupae and 8 dead adults of an ectoparasitoid identified as Pseudotorymus sp.<sup>2/</sup> (Hymenoptera: Torymidae) were also found, but it is not clear how many larvae were parasitized by this insect.

The results of the routine dissections of buds and galls generated the following information: In April only buds were present; eggs were collected on bud samples collected on April 19, May 2 and May 11; the highest number of eggs was found on samples collected on April 19 with  $29.42 \pm 10.46$

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<sup>1/</sup> Identified by Dr. P.M. Marsh, Systematic Entomology Laboratory, USDA, ARS

<sup>2/</sup> Identified by Dr. E. Grissell, Systematic Entomology Laboratory, USDA, ARS

eggs/bud. The first galls were found on May 2; and the majority (80%) of them were still in the early stage. On July 10 only mature galls were found and they contained the lowest number of larvae/gall. Half of them had no larvae and 14 contained pupae or adults. In one gall a single individual of a Torymid ectoparasite was found. The results of these dissections are presented in Table 3.

In the inspections conducted in June and July it was observed that the mature larvae of D. capsulae exit from the galls and fall to the soil early in the morning, or just after a rain, when the humidity is high.

The biometric data of D. capsulae are summarized in Table 1. The mature galls (n = 25) had the following mean sizes: length  $8.36 \pm 1.51$  mm. and width  $5.06 \pm 0.95$  mm.

June is the best time to evaluate the actual infestation due to D. capsulae because the majority (95%) of the galls have reached maturity and at that time they were 23% infested. In the samples collected in May, the percentage of infestation appeared to be lower, but this was probably due to the fact that in May the majority of the galls (ca. 80%) were in the early stages and some of the infested ones escaped being counted.

From the information obtained in Laboratory and field observations we learned quite a bit about the biology of the midge. The midge is univoltine and since the eggs were found in our observations from April 19 to May 11 we assume that the overwintering adults first appear about mid-April and continue to emerge from the soil until mid-May. We observed that the adults, which live 2-4 days, lay eggs in groups on the inner part of the bracts of the flower-buds of E. esula, and in the field from 9 to 40 eggs/bud were found, but in the laboratory females layed up to 129 eggs/bud. Eggs held in hatching containers and kept in the laboratory, hatched in 3-4 days. Under natural conditions, the newly hatched larvae move into the cyathium and feed either on

the inner walls of the cyathium or on the floral organs. The larval feeding or presence causes a gall to be produced by deformation and enlargement of the cyathium. Larvae complete their development in 4-5 weeks then early in the morning or after a rain when the humidity is high the mature larvae leave the galls, fall to the ground, penetrate the soil, and go into diapause until the following spring.

#### Host Specificity

The results of the preliminary tests demonstrated that some North American biotypes of leafy spurge are suitable hosts. On leafy spurge from Montana, in one replicate 75 eggs of D. capsulae were found distributed among 3 flower-buds. In the other 3 replicates, 2, 3, and 4 mature galls respectively of this midge were formed, with a range of 8-15 mature larvae/gall. In one replicate with leafy spurge from Oregon, 65 eggs were distributed between 2 flower-buds while a later examination of the two other replicates disclosed 3 and 4 mature galls with a range of 5-12 mature larvae/gall. Lastly on E. esula from S. Rossore, Italy, which served as control, 150 eggs were laid in 2 replicates, distributed over 5 flower-buds. In the remaining 3 replicates, 4, 2, 2 mature galls respectively were developed with a range of 4-15 mature larvae/gall.

To conduct extensive tests in 1984, massive collections of mature larvae of D. capsulae were made both at S. Rossore (Pisa) Italy on E. esula, and in eastern Hungary on E. virgata.

Table 1. Biometric data of Dasineura capsulae<sup>1/</sup>.

	Sample size	Length (mm)			Width (mm)		
		$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD
Eggs	40	0.27	$\pm$	0.02	0.07	$\pm$	0.01
Larva (I instar)	15	0.84	$\pm$	0.18	0.16	$\pm$	0.04
Larva (II instar)	15	1.90	$\pm$	0.22	0.40	$\pm$	0.04
Larva (III instar)	15	2.72	$\pm$	0.39	0.61	$\pm$	0.10
Adult ♀	10	2.32	$\pm$	0.09	0.41	$\pm$	0.02
Adult ♂	10	1.69	$\pm$	0.06	0.41	$\pm$	0.02

<sup>1/</sup> Magnifications used: eggs 50 x, I instar larvae 40 x, II and III instars 25 x, adults 40 x

Table 2. Periodic counts of flower-buds, flowers and Dasineura capsulae galls on 20 plants of Euphorbia esula L. at S. Rossore (Pisa) Italy<sup>1/</sup>.

Collection Date	No. of flower buds/plant			No. of flowers/plant			No. of galls/plant			% infestation <sup>1/</sup>
	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	$\bar{X}$	$\pm$	SD	
May 15	51.15	$\pm$	41.16	68.60	$\pm$	34.50	5.95	$\pm$	5.73	8.0
May 26	4.80	$\pm$	17.23	57.85	$\pm$	30.38	8.35	$\pm$	8.16	12.6
June 15	1.55	$\pm$	2.80	92.30	$\pm$	36.57	31.71	$\pm$	22.06	23.4

<sup>1/</sup> % of infestation was calculated on the total number of flowers and galls only.

Table 3. Periodic dissections of flower buds and Dasineura capsulae galls collected on Euphorbia esula, at S. Rossore (Pisa), Italy.

	No. of infested flower buds <sup>1/</sup>	$\bar{x}$ + SD eggs/bud <sup>2/</sup>	$\bar{x}$ + SD larvae/gall <sup>3/</sup>	No. of parasitoids ( <u>Pseudotorymus</u> sp)	Number of empty galls
April 2	0	0	Galls were not present		
April 19	12	29.42 + 10.46			
May 2	15	25.87 + 12.81	13.56 + 7.23		
May 11	5	14.40 + 6.31	11.80 + 5.56		
May 26	0		12.28 + 6.40		4
June 15			9.26 + 7.57	9	12
July 10			4.90 + 6.27	17	25

<sup>1/</sup> Based on sample of 100 flower-buds

<sup>2/</sup>  $\bar{X}$  + SD eggs/bud was calculated on the number of infested flower-buds.

<sup>3/</sup>  $\bar{X}$  + SD larvae/gall was calculated on a sample of 50 galls.



APHTHONA ABDOMINALIS

Pasquale Pecora

A flea beetle, Aphthona abdominalis (Duftsch.) (Coleoptera: Chrysomelidae), found and collected at Pisa on Euphorbia esula and identified by R. E. White, Systematic Entomology Laboratory, IIBIII, USDA, was taken under study as candidate agent for the biological control of leafy spurge (E. esula L. "complex") in North America. Significant defoliation of the host plant is caused by adult feeding, however, the impact of larval feeding is not known.

A. abdominalis is distributed in Europe and there are literature records of A. abdominalis from the following plants; Euphorbia cyparissias L., E. paralias L., E. seguierana Necker, E. stricta L., and Linum sp. Only one of these plants, Linum, is of economic importance, and this record is questionable and needs verification.

Biology Notes - We first observed adults of A. abdominalis in early April at S. Rossore (Pisa) Italy. Adults of this insect continue to be found in the field throughout September. Two stocks of adults collected on June 15 (30 individuals) and September 2 (20 individuals) respectively on E. esula, kept in the laboratory in plexiglas cages (10/cage) provided with a bouquet of leafy spurge, produced eggs. The small yellowish eggs were laid in groups of 4-5 on the cotton at the base of the bouquets. A sample of 50 eggs kept at ambient outdoor temperature, required 7-10 days to hatch and 38 (76%) of them were fertile.

Host Specificity - Adults of A. abdominalis emerged from of E. esula plants collected in April 1983 (300 plants), for laboratory needs. At the end of August the first flea beetles were observed on leafy spurge plants in the

laboratory garden, the number of adults increasing progressively during September. All these plants were in pots, in a plot 10 m x 10 m and the aerial portions of both Italian and American taxa of leafy spurge were heavily damaged.

Since adults of A. abdominalis were available, a preliminary feeding test was started on September 15, 1983 on a restricted number of plants in order to have some indication on the host plant spectrum of this flea beetle. The insects (20 adults/cage) were exposed to potted test plants, which were caged in transparent plastic tubes (20 cm diam; 60 cm. height) with organdy covered holes in the side and and organdy top for air circulation. Sixteen test plants and a control (replicated once) were included in this experiment. The plants were left undisturbed for 20 days then the damage on plants was evaluated, and numbers of dead and living insects were recorded.

The following are the results of this test:

- a) Control: E. esula S. Rossore, Pisa, Italy - 8 adults alive, plants completely defoliated.
- b) E. esula "complex", US leafy spurge acceptance test: Montana and Oregon varieties: plants completely defoliated 10 and 12 adults were alive.
- c) E. marginata, E. lathyris: 60-70% of the aerial part completely destroyed; all insects dead.
- d) E. milii: feeding observed only on flower-petals; 20% of flowers heavily damaged; 3 adults were still alive.
- e) E. tirucalli: 30% of young leaves heavily damaged; 5 adults still alive.
- f) Ricinus communis: two leaves were lightly damaged; all insects dead.

g) E. heterophilla, Poinsettia pulcherrima, Manihot palmata,  
Mercurialis annua, Linum flavum, Nerium oleander, Ficus elastica  
Salvia splendens, Zea mays: No feeding. All insects dead.

The results of this preliminary test demonstrated that adults of A. abdominalis fed only on plants in the genus Euphorbia and remained alive only on the control and the closely related American biotypes of leafy spurge included in the test, except for those that fed on the flowers of E. milii and the ephemeral leaves of Euphorbia tirucalli.. Lastly, no feeding was observed on Linum flavum which corroborates our suspicion that this host record was mistaken.



BAYERIA CAPITIGENA

Pasquale Pecora

Bayeria capitigena (Bremi) (Diptera: Cecidomyiidae) is a multivoltine gall midge associated with Euphorbia spp. in Europe, whose larvae form meristematic tip galls in leafy spurge thus reducing seed production. B. capitigena host specificity studies were carried out in 1982, and the results of these studies indicate that this midge is able to oviposit and complete its life cycle only on plants of the genus Euphorbia (subgenus Esula).

In 1983 B. capitigena was approved for introduction into quarantine at Albany, CA. for final testing. In order to ascertain the suitability of the egg stage for shipments to the US a study was conducted to determine the longevity of Bayeria eggs under several conditions where time, substrate, and moisture were varied.

On May 11, 1983, 30 Bayeria galls were collected on E. esula at S. Rossore (Pisa), Italy. Adult midges emerging from these galls started to lay eggs in the laboratory on May 20. Samples of Bayeria eggs, left undisturbed on the meristematic tips, were placed in different kinds of hatching containers with moistened cotton on plaster of Paris to provide adequate humidity, and stored in a refrigerator at 4°C for 3-10 days then transferred out of doors for hatching.

The results are as follows:

Hatching container	No.days in refrigerator	No.eggs used	No.eggs hatched	% hatch
Moistened cotton	7	100	70	70
Dry cotton	7	409	328	80
Plaster of Paris(moist)	7	104	78	75
Dry cotton	10	422	132	31
Moistened cotton	3	112	59	53

These results indicate that eggs stored in refrigerator for 7 days are still fertile (70-80%) therefore B. capitigena eggs in appropriate containers kept at low temperature during shipment by using cold-packs (from cold retaining material in sealed plastic containers), can be sent successfully to the quarantine in the US.

In order to provide insects with a better possibility of establishment in the colder, northern states, an attempt was made to establish a colony of cold-hardy strain of B. capitigena at the Rome Laboratory. To start this colony 300 galls from E. virgata were collected in Eastern Hungary, in mid-June 1983, but the attempt failed because the larvae of this midge were so heavily parasitized that the establishment of a colony was not possible.

Work Plan for 1984

Bayeria capitigena

Massive collections of Bayeria capitigena from E. esula will be made at S. Rossore (Pisa), Italy, and shipped to Albany, Ca. for final testing. Bayeria galls will also be collected in the Balkans earlier in the year in a second attempt to establish a colony of a cold-hardy strain of B. capitigena at the Rome Laboratory. This colony will serve to conduct some trials at the Rome Laboratory, as well as provide eggs for shipment into quarantine at Albany, California.

Dasineura capsulae

Extensive testing will be carried out in both the field and laboratory in order to determine the degree of host specificity of this midge.

Aphthona abdominalis

Extensive testing will be conducted in the laboratory to determine the degree of host specificity of this flea beetle. Biological studies in both the field and laboratory will be continued.



EUPHORBIA Spp.

R. Sobhian

Thessaloniki, Greece

In 1982, seed samples of 5 Euphorbia "esula" varieties were provided by the USDA BioControl of Weeds Laboratory in Albany, CA., to be tested with Simyra dentinosa (Lepidoptera: Noctuidae) which attacks Euphorbia seguieriana in Greece. The seeds originated in Montana, Idaho (Fremont, Co.), Nebraska (Davis, Co.), Canada (Camloops B.C.) and Canada (Jameson Sask.). The seeds from Montana were planted on May 21 and the rest of the seeds, which were sent later, were planted on June 9, 1982. Also, a sample of E. esula root cuttings, originating in Montana, was shipped from Albany, California and arrived on Oct. 13, 1982, in good condition. In order to prevent the plants from escaping all the seeds were grown in pots and kept on an asphalt road to prevent their roots from penetrating into the ground and the plants were prevented from forming seeds by removing their flower buds. All the E. esula plants were destroyed after the feeding test with Simyra ended.

The origin of the insects for the test was from 160 Simyra larvae collected from E. seguieriana near Volvi lake (east of Thermi) which allowed to pupate in a 30 x 30 x 30 cm screen cage (among dried food plant stems and leaves and on cage walls). During the winter the cage containing most of the pupae was kept outdoors, but a part of the pupae were kept in refrigerator at 8°C. On March 21, 1983, the first 10 adults emerged in the cage outdoors. The cage containing these adults along with the rest of the pupae were placed under a 130 x 80 x 70 cm screen cage with 10 potted E. "esula" plants (3 from Montana, and each of the other 4 varieties). The door of the small cage was opened so that the existing adults and the emerging ones could move freely to the leafy spurge plants in the larger cage.

To serve as a control, 5 field collected E. seguieriana plants were potted and placed under a cage of the same size and type used for the "esula" plants on the following day (March 22). About half of the Simyra pupae and half of the adults were transferred into this cage, so the present and emerging adults would have access to the Seguieriana plants for oviposition.

Sugar water and sugar water-yeast hydrolysate were offered as food to the adults in both cages, but no feeding was observed. The insects are not good flyers and sit for hours on cage walls or on a plant with no activity. No mating was observed.

After 20 days, on April 11, when no more living adults could be seen in the cages, the plants were removed from the cages and examined for eggs. Only one patch of about 40 eggs was found on an "esula" plant (var. Nebr.) and no eggs were found on the Seguieriana plants which were in bad condition suffering from the transplanting shock. The first instar larvae that emerged from the eggs on the "esula" plant fed and completed their development to pupae on E. "esula".

In another trial field collected Simyra larvae, in different instars were transferred from E. seguieriana to E. esula varieties in order to find out whether they would accept the new host plants or not. They accepted them readily.

Also, field collected eggs from E. seguieriana were allowed to hatch on Euphorbia pulcherrima for a 1st instar larval feeding test. The larvae nibbled a little on the leaves without making the characteristic webbing at the apex of the plant and died without molting. Poinsettia is an unsuitable host for Simyra dentinosa.

The pupae kept in the refrigerator were taken out on March 23 and kept in laboratory. The first adult emerged 13 days later on April 5. Two

females of this group were dissected the first day after they emerged. One female had 350 eggs in her ovaries, and the other had 85.

In the field the disc-like eggs are laid, in more or less regular rows, on the underside of leaves, in masses ranging from 61-242 the mean number of eggs in 28 masses was 131.1 eggs/mass. The eggs hatch in 10-11 days under natural conditions, and some of the field collected eggs showed evidence of parasitism, but no parasites were reared from the eggs.

The larval development takes about 30 days from egg to pupa and there are 6 larval instars. Larvae in the 1st to 5th instars are gregarious, spinning a web on the top of the plant and feeding in the webbing. They become solitary in the 6th instar when they leave the plant and wander off to pupate. When the larvae were forced to pupate in crowded cages, 1-3 insects were found in a common cocoon, some pupated and some dead. The adults resulting from these pupae were often malformed, mainly having crumpled wings. Table 1 shows the approximate body length, width of the head capsule and the time passed at each larval instar at ambient temperatures.

Table 1. Simyra dentinosa, approximate body length, head capsule width measurements and duration of larval stages at ambient temperature.

Larval instars	:	L1	:	L2	:	L3	:	L4	:	L5	:	L6
	:		:		:		:		:		:	
No.of days from molt	:		:		:		:		:		:	
to molt	:	6	:	5	:	4	:	5	:	5	:	5
Approximate body	:		:		:		:		:		:	
length in mm	:	1.5-2	:	4.5-5	:	9	:	15	:	22	:	20
Approximate width of	:		:		:		:		:		:	
head capsules in mm	:	0.428	:	0.624	:	0.95	:	1.4	:	2	:	3.2

CENTAUREA SOLSTITIALIS

R. Sobhian

Thessaloniki, Greece

a) Urophora siruna-seva (HG)

In safflower (SF) seed heads dissected in 1982 a gall formed by an unknown Urophora sp. (Trypetidae) was found. It was assumed that it was a Urophora macrura gall since U. macrura attacks wild safflower Carthamus lanatus in the area. In order to confirm which Urophora attacks safflower, (Carthamus tinctorius) in Thermi, about 50 SF plants were grown in the same location as a 1982 planting to also serve as hosts for any interested U. macrura in the field population. In addition, on May 27 a cage trial, replicated 3 times was set up caging five pairs of U. macrura on YST and 3 pairs on SF. On July 26 all ripe seed heads in the cages were collected and examined for Urophora galls. The results are shown in Table I. To complete the study specimens of the U. macrura collected on Carthamus lanatus and Carthamus sp. for use in the experiment were sent to Prof. Helmut Zwolfer, at the University of Bayreuth, Bayreuth, West Germany, for identification. He confirmed the identity of the insects used in the test to be U. macrura.

Table I. Infestation of SF and YST by U. macrura and U. siruna-seva caged on plants.

Flies:	<u>U. macrura</u>		<u>U. siruna-seva</u>	
	YST	SF	YST	SF
Plants				
Examined No. Heads	74	34	74	32
No. Galls	-	45	10	-

Safflower was attacked only by U. macrura and YST was attacked only by U. siruna-seva.

In addition to the above experiment, U. macrura females were observed ovipositing on safflower flower buds, on May 20, in Thermi. They lay their eggs in clusters between the bracts of relatively young buds. One bud in which oviposition occurred was dissected, disclosing a cluster of 14 eggs. Six other buds, in which U. macrura females made oviposition attempts, were labeled on May 30 and dissected on July 1 and 4. Six galls were found in 3 of those seeds heads (2, 3, and 1 gall/head).

Wild U. macrura and U. siruna-seva adults were collected and released on SF and YST plants, grown near to each other (0-1,5 meter apart), at the University farm, where the laboratory is situated. In daily examinations U. macrura was found only on SF and U. siruna-seva was found only on YST.

The safety of the Greek biotype of U. siruna-seva was confirmed by these tests so the decision was made to release it in California in the spring of 1984.

To provide insects for release, we dissected 28,595 YST seed heads during September and October, and collected 1108 U. siruna-seva galls, which were mailed to Albany, CA. for fly emergence, freeing of parasites and liberation. Sampling showed that about 50% of the galls were parasitized but we did not separate out the parasitized galls, because we were afraid we would damage the unparasitized fly larvae by opening all the galls to check for parasitism.

b) Bangasternus orientalis and Bangasternus spp.

It was decided to study the biology of B. orientalis in more detail. But since B. provincialis and B. planifrons occur in our area along with B. orientalis some information was collected on these species as well.

Over 20 weevil specimens reared from YST seed heads and 6 reared from Centaurea calcitrapa seed heads were mailed for identification.

#### Oviposition Fertility and Fecundity

In order to determine the longevity and fecundity of B. orientalis, 7 pairs were caged with YST bouquets in 550 cc paper cups, with plastic tops. Holes, (5 cm diameter) were cut in the plastic tops of the cages and covered with gauze to permit an exchange of air.

The experiment was started on May 11 and the bouquets were replaced daily. The number of eggs laid by each female was recorded and the bouquets on which the eggs were laid, were kept in the laboratory and checked for egg hatch. It was also noticed that the insects lay eggs both day and night.

Since B. orientalis adults copulate repeatedly during their whole oviposition period, a second experiment was started using the same material and methods, but instead of a pair, only one mated female was caged in each cup. The purpose of this second experiment was to determine the number of eggs laid by single female and also to determine how long a female would lay fertile eggs after the last copulation.

The single females laid more eggs than females in the paired experiment and they also laid more fertile eggs during the whole period of the experiment, showing that repeated copulation is not necessary for the production of fertile eggs during the oviposition period. From 974 eggs laid by pairs, 573 eggs hatched (58.83%) and from 1332 eggs laid by single females, 732 hatched (54.95%).

Nine of the females that died in the oviposition tests were dissected. Three had no eggs left in their ovaries while the ovaries of the other 6 females contained varying numbers with a maximum of 2 developed and 9 undeveloped eggs.

#### Location of eggs on the plant

Normally, after the B. orientalis female deposits an egg, she covers the egg with a cap of black substance resembling fecal material that is issued from the posterior part of her abdomen. This egg cap is generally tear shaped, forming a sort of tail when she pulls away from the site.

For oviposition, the females select young floral buds (BU-1 and BU-2 of Maddox 1981), but occasionally they oviposit on the leaves near the tips of branches without well defined buds. Out of 308 field collected eggs only 6 were laid on the stems, near the buds, one directly on a flower head and the rest of them were laid on the lower sides of the small leaves, adjacent to the forming flower heads.

When no YST buds were available, females in captivity laid eggs without an egg cap on the other plants available.

In order to study the larval development, 38 adults were caged for 4 days on both May 27-28 and June 7-8 on 5 YST plants growing in the garden. In this way we obtained several hundred of eggs, which were laid on plants outdoors, on known dates. Of 469 eggs, which were examined between June 3 - July 25, only 25 hatched, 281 were parasitized, 53 were dried out and 130 did not hatch for unknown reasons, probably because they were sucked dry by predators. Because of this high egg mortality, it was not possible to follow the larval development of the weevil.

In a later experiment, 5 adults were caged for one day, on a potted plant in the laboratory (June 30) and 10 eggs were laid on the plant. The plant was then placed outdoors under natural conditions. The flowers on whose buds these eggs were laid were checked on August 3. Nine eggs had hatched, and only one living last-instar larva, resting in its cocoon, was found in one flower head which was in the seed formation stage. The experiment shows that

the larva develops, from egg to pupae, in about 35 days. The rest of the larvae were found dead, either inside or outside the dried flower heads. The eggs may have been laid on older buds and the larvae died not having tender developing achenes to feed on.

The examination of about 20 larvae, dissected from various samples show that there are probably only 3 larval stages. The L1 larvae are yellowish, straight, with dark brown head capsules. L2 and L3 larvae are lunate, with a milky color. Normally there is only one adult or larva in each seed head, occasionally however, two are found. This presence of 2 insects in a single seed head would probably be more common if egg mortality was not so high.

#### Mortality Factors

Natural mortality of the immature stages of Bangasternus is very high, especially egg mortality. Nine egg samples (N = 485) were examined from the same location in Thermi, from May 22 to August 13 and the egg mortality ranged from 38.7% - 98.7%.

Table 3 shows the results of this examination. The eggs that are categorized as "not hatched" are eggs that were not parasitized, not dried out and not eaten by a mandibulate predator. It seems that these eggs may have been killed by a sucking insect because examination disclosed that only the white, thin chorion of the egg remained under the egg caps. Eggs killed in this way were found only in June.

The eggs collected May 22 - June 29, were collected along with young buds of about equal size, so the eggs in each sample were about the same age at the time of collection. The eggs in the sample of July 31-August 15 were collected along with seed heads in the seed formation stage. In the August 15 sample, 61.3% (n= 189) of 308 eggs collected, had hatched but only 10 larvae; 26 pupae and 13 adults were found, (all living) making a total of 49 living B.

orientalis that were left from 189 eggs that were hatched. If the 10 larvae and 26 pupae would develop to adults, which normally is not the case, the total mortality from egg to adult would be at least 84%. Sometimes the total mortality is higher than this, for example in the eggs examined June 28 the egg to adult mortality reached 98.7%.

In another late season collection (July 16), 100 ripe YST seed heads with at least one egg on them were collected in Kozani. Out of 118 eggs found on these heads only 5 living larvae, 4 pupae and 3 adults were found. If all these pupae and larvae would have developed into adults, the total mortality would have been 89.83%.

#### Natural enemies of adult B. orientalis.

In 1982, 14 females and 12 males were dissected and one female and one male were "parasitized" by pyemotes mites located under the elytra. In 1983, 34 females and 9 males were examined but no mite infested individuals were found.

#### Key for egg determination

Since 3 species of Bangasternus (orientalis, provincialis and planifrons) occur near Thermi, it is necessary to be able to determine the eggs of each species so when we work with field collected eggs we know that we are working with the correct species.

When the female of Bangasternus orientalis or B. provincialis oviposits, she covers the egg with a black excrement (perhaps feces) and as she moves away from the egg cap the black material is pulled to a point.

We examined 426 field collected eggs of B. orientalis and of these eggs 94.8% were laid on the lower side of the leaves (one egg on upper side), 4.92% on stems and only one egg directly on a flower head. The pointed part of the dark egg cap is normally downward. Of the 425 eggs, which were laid on leaves and stems, only 7 (1.6%) eggs had the stalk of the egg cap upward and

those 7 eggs were laid on the stem.

Field collected eggs (n = 123) of B. provincialis were also examined, and over 60% of these eggs were laid on stems and the rest were laid on leaves with 89.4% of the pointed part of the egg caps directed upward.

The egg cap of B. planifrons has no pointed part and so it is very easy to distinguish its eggs from the eggs of the other 2 species. Also, B. planifrons eggs were found only on bracts of Carthamus lanatus and another Carthamus sp. It will also oviposit and reproduce on C. tinctorius, the cultivated safflower.

The results of these observations along with other morphological characteristics of the egg caps resulted in the following key for the determination of the eggs of the 3 species.

- A. Eggs directly on bracts, egg cap without stalk mean length 0.75 mm x 0.62 mm wide (max 0.84 x 0.64 min 0.64 x 0.52)

B. planifrons

- B. Eggs mainly on leaves, egg cap with stalk normally extending downward. Mean egg measurement 0.66 mm long (max. 0.92 mm long x 0.72 mm wide, min. 0.80 mm long x 0.60 mm wide). The mean egg cap width is 0.86 mm and lower side of the egg cap is thick, opaque and if removed from the plant the egg is not visible inside.

B. orientalis

- C. Eggs mainly on stems, egg cap with stalk, normally extending upward. Mean egg measurement is 0.60 mm long x 0.47 mm wide

(max 0.68 x 0.48 mm., min 0.56 mm x 0.44 mm)

The lower side is thin, more or less transparent. If removed from the plant the egg can be seen inside the cap.

B. provincialis

Also, there is no size overlap between B. orientalis and B. provincialis eggs, the latter are much smaller, so they can be separated. However, both these species overlap with the size of the B. planifrons eggs, but the absence of a point on the egg cap easily separates the eggs of this insect from the eggs of the other two species.

Effect on Host plant

The young B. orientalis larva feeds for several days outside the flower heads on the parenchymal tissue of stems, and the mesophyll tissue of leaves and bracts, until it enters to the central part of a flower head, where it feeds on achenes, reducing their numbers. A sample of seed heads (n = 1229), with at least one hatched egg on them, were measured, dissected, and categorized in 7 classes, depending on their sizes, from 6-9 mm diameter. Only 84 seed heads contained pupae or adults and the average number of seeds left in these heads were compared with the average number of seeds in non-infested heads. 10 uninfested heads from each of 7 size categories were dissected and the mean number of seeds in the heads of each category were recorded. Over 300 seed heads were measured and dissected in order to find 10 seed heads in each category without infestation.

In the 6 mm diameter seed heads 10 out of 42 were not infested, in the 7 mm seed heads 10 out of 71 were not infested, in the 9 mm seed heads 10 out of 23 seed heads were not infested. Table 2 shows the results of this study.

Table 2. Average number and percentage of seeds destroyed by one B. orientalis larva in various sizes of heads.

Seed head Diameter in mm.	6-6.4	6.5-6.9	7-7.4	7.5-7.9	8-8.4	8.5-8.9	9+	Total
No. Infested heads counted for seeds	8	9	27	15	15	6	4	84
Mean undamaged seeds in infest- ed heads	4.25	11.55	10.62	15.33	16.73	29.16	37	17.80
Mean No. seeds in uninfested heads (N = 10)	20.6	26.7	49.3	45.1	60.1	85.8	89	53.8
Calculated % of seeds destroyed per category	79.4	56.7	78.4	66	72.2	66	65.1	66.9



CHAETORELLIA cf. HEXACHAETA

Rouhollah Sobhian

Flies in the genus Chaetorellia (Diptera: Tephritidae) were studied by Professor H. Zwolfer in the 1960's to determine their potential as biocontrol agents of Centaurea solstitialis (YST). An account of this work can be found in Report No. 6, May 1968, of the Commonwealth Institute of Biological Control.

Preliminary studies on Chaetorellia sp. attacking C. solstitialis in Greece were undertaken in 1983 to develop some baseline information on the bionomics, reproductive behavior and capacity, and host specificity of a Greek population of Chaetorellia hexachaeta (identified by H. Zwolfer).

Materials and Methods

The adults for the studies came from three sources: 1) most were reared from YST heads collected in Thermi, Greece; 2) some were collected directly from YST plants in Thermi; and 3) a few were reared from YST heads collected in Kozani, Greece. Yellow starthistle plants growing wild in Thermi were used as test material; however, cultivated safflower (Carthamus tinctorius) was grown from seed.

The method employed to collect the host specificity data involved caging adult flies with whole plants, branches, or small flower bud bouquets of the test plants. Flower heads were collected at the end of the season and examined for Chaetorellia and Chaetorellia induced seed damage.

Cages were provided with cotton plugged vials with water. Flies obtained water from these vials and protein from strips of paper coated with a dry layer of yeast and sugar. In one laboratory study bouquets of YST flower

buds (held in water filled bottles) were offered to ovipositing females in cages. Each bouquet was comprised of one Bu3, Bu4, and Bu5 YST flower bud (according to Maddox 1981). Exposed buds were replaced daily with new ones and then examined under a microscope to count the number of eggs in or on them. Seven cages received one freshly emerged male and female. Males that died before females were replaced. One female was mated once and then put into a cage by herself. This study also provided data on adult longevity and female fecundity.

Another test, (preliminary no-choice oviposition) was run by exposing a varying number of flies to bouquets of several test plants in laboratory cages. Some of these flies were at least 40 days old so the results are indicative only. Another small test measured the oviposition response of flies to heads of field grown C. solstitialis and C. tinctorius. Here, 2-5 pairs of flies were field caged with branches of the two kinds of plants.

A third study was conducted to record the response of flies to C. solstitialis alone, C. tinctorius alone, and the two plants together. Bouquets were offered to flies in laboratory cages and behavioral responses were recorded every 10-15 minutes.

Lastly, Chaetorellia infested and uninfested seed heads of C. solstitialis were field collected to evaluate the extent of damage to the seeds caused by the fly.

Table 1. Synoptic table showing data collected in cages containing one pair of Chaetorellia hexachaeta flies and three bud stages of yellow starthistle. Greece 1983.

Flower bud	Number of Eggs Laid in 8 Cages							
Stage	1	2	3	4	5	6	7	8 <sup>1/</sup>
Bu3	48	17	2	6	18	33	39	3
Bu4	178	49	8	36	38	167	80	20
	(12) <sup>2/</sup>	(11)	(4)	(11)	(11)	(13)	(6)	(5)
Bu5	17 <sup>3/</sup>	0	4	0	6	6	3	0
Female Longevity								
in days:	60	51	8	9	9	40	35	31

<sup>1/</sup> This cage contained one female which was mated only one time.

<sup>2/</sup> Values are maximum number of eggs laid on Bu4 stage buds.

<sup>3/</sup> Values are the maximum number of eggs laid per female per day on Bu5 stage buds.

Close observation of flies in the cages also revealed the following:

- 1) adults copulate frequently;
- 2) time of copulation is very variable (20 to 100 minutes);
- 3) copulation seems to stimulate oviposition behavior in females;
- 4) young adults (24 h old) will copulate and females will oviposit the same day they mate;
- 5) eggs are deposited between the last rows of the bracts;
- 6) one or several eggs are layed at one time;
- 7) oviposition peaks about 10 days after emergence;
- 8) some females will oviposit each day they

live, others stop a few days before they die; 9) eggs are fusiform, white with stalks about twice as long as the egg. According to Zwolfer, the eggs of other Chaetorellia species do not have stalks.

Eggs hatched within 2-4 days in the laboratory (25-27°C and 70-88% RH). Neonate larvae fed a little on the edges of one or two bracts before burrowing into the bud. Once in the bud a larva feeds on achenes, one after another, until it pupates. Last instar larvae of the first generation form loose cocoon almost parallel to the long axis of the flower head. Second generation larvae, in contrast, form a dense cocoon for overwintering; this cocoon is positioned almost perpendicular to the long axis of the flower head.

The following data were obtained in no-choice oviposition tests conducted in the laboratory.

<u>Test Plant</u>	<u>Number Eggs Laid over Time</u>
<u>Carthamus tinctorius</u>	No eggs in 6 days
<u>Cirsium</u> sp.	43 eggs in 3 days
<u>Zinnia elegans</u>	14 eggs in 4 days
<u>Scolymus hispanicus</u>	No eggs in 3 days
<u>Carlina corymbosa</u>	6 eggs in 4 days
<u>Carthamus lanatus</u>	No eggs in 2 days
<u>Centaurea</u> sp.	15 eggs in 2 days
<u>Carthamus</u> sp.	No eggs in 2 days

Note: Most of the cages contained 15 females and 6 males.

There was no indication that the flies accepted C. tinctorius when they were field caged in a June 4 test with branches of this plant. However, 3 egg shells, evidence of larval feeding, 1 pupa, and 5 pupal exuviae were

found in the heads of yellow starthistle control on July 13. The same pattern was seen when yet another series of flies were caged with these plants on July 11. This time, 175 egg shells, 32 larvae, 5 pupae, and 5 pupal exuviae were found when the heads on YST were dissected on August 7.

Table 2 summarizes the behavioral responses of flies to safflower and yellowstar thistle.

Table 2. Behavioral responses of Chaetorellia flies to safflower and yellow starthistle, cage study, Greece 1983.

Observation	Test 1 <sup>1</sup>					:	Test 2 <sup>2</sup>	
						:		
						:		
	Cage 1		Cage 2		Cage 3	:	Cage 1	Cage 2
	(SF)		(SF + YST)		(YST)	:	(SF)	(YST)
No.alighting females	1	1	0	12	9	:	11	29
No.alighting males		1	0	16	4	:	5	22
No.copulating pairs		0	0	6	0	:	1 <sup>3/</sup>	2
No.of ovipositions		0	0	0	1	:	0	4

1/ Each cage had 3 pairs of flies and bouquets were examined every 15 minutes for 3 hours and 15 minutes.

2/ Each cage had 4 pairs of flies and bouquets were examined every 10 minutes for 2 hours and 30 minutes.

3/ Pair left after 5 minutes.

Table 3 shows that Chaetorellia larvae are responsible for considerable seed destruction in seed heads of different sizes.

Table 3. Average number of seeds recovered from Chaetorellia infested and uninfested seed heads of yellow starthistle.

Type of seed heads	Diameter (mm) of Seed Head				
	6	6.5	7	7.5	8
Average No.seeds per each size head					
Infested Head	3.16(6) <sup>1/</sup>	5.85(7)	6.16(6)	3.6(5)	9.75(4)
Uninfested Head	20.6(10) <sup>1/</sup>	26.7(10)	449.3(10)	45.1(10)	60.1(10)
%Seeds destroyed	84.7	78.1	87.5	92.0	83.8

<sup>1/</sup> Numbers in parenthesis denote sample size.

### Results

Table 1 summarizes the results where flies were offered YST buds of different stages. The data indicate: 1) flies prefer Bu4 flower buds, followed by Bu3 and Bu5 buds; 2) females can live up to 60 days and lay 243 eggs during this period.

Table 2 shows the adult flies have little interest in safflower when exposed to the plant in a no choice situation and even less interested when exposed in a cage that also has YST.

Table 3 shows that the larvae of Chaetorellia do severe damage to infested YST heads of all sizes examined (6 mm to 8 mm diameter). The mean percentage of seeds destroyed in all the head sizes measured  $N = 78$  heads was 85.22%.



CENTAUREA SOLSTITIALIS

Clement, Cristofaro, Mimmocchi

We were assigned to the Yellow starthistle (YST) project in late 1982 and given responsibility for determining if the rosette and root inhabiting Apion weevils had potential as biocontrol agents of the target weed in North America. Some useful information was obtained on these weevils and other natural enemies of YST and related plants.

A second study was started in 1983 to determine if field plantings of test plants at the Rome Laboratory would be useful in identifying the host utilization patterns of candidate biocontrol agents. These plants should also serve as a "trap crop" for a diversity of natural enemies.

The Apion and field plot studies will continue in 1984

Apion spp.

Research objectives were: 1) To conduct a literature search for host records of Apion, subgenus Ceratapion; 2) To obtain a good series of Apion adults from YST and related plants for taxonomic purposes; 3) To locate YST infestations in central and southern Italy and determine if Apion attacked the plants; 4) To study the life history of the rosette and root inhabiting Apion in YST; 5) To conduct preliminary host specificity tests. No progress was made under objective 5. Accomplishments under the first 4 objectives are summarized below.

### Objective 1

A review of the literature turned up no records of A. alliariae Herbst on crop plants (Review of Applied Entomology, Series A, 1913-1982; Zoological Record 1950-1971; Grandi, 1951; Della Beffa, 1961; Hoffmann, 1958; Balachowsky, 1963). Emphasis was placed on this species as it was felt by other biocontrol workers that it might be commonly associated with YST.

There are several records for A. (Ceratapion) carduorum Kirby attacking young artichoke plants, Cynara scolymus L. (see Poinar et al. 1980). We are unaware of any other crop plant records for the Ceratapion. Cirsium, Carduus and other thistle genera are in the literature as host plant of the Ceratapion so other thistles will have to be represented in the host plant test list.

Host records (Table 1) for the Ceratapion were compiled from five sources: 1) Hoffmann, A. 1958. Faune de France, Coleopteres Curculionides, Vol. 62 pp. 1512-1521; 2) Zwolfer, H., 1965. Preliminary list of phytophagous insects attacking wild Cynareae (Compositae) in Europe. CIBC Tech. Bull. No. 6, pp. 81-154; 3) Dr. D. Whitehead's (Research Entomologist, Systematic Entomology Laboratory, USDA) remarks on Apion submitted for identification by P. Dunn in 1981; 4) Dr. Whitehead's remarks on Apion submitted for identification by Dr. S. Clement on August 5, 1983; 5) Steklova, M. 1983. K. Poznanin Druheho Zlozenia A. Ziunych Rastlin Podcelade Apioninae (Coleoptera: Curculionidae) V. Jurskom Sure., Biologia (Bratislava), 38 (2): 1239-144.

The taxonomic alignment in Table 1 follows Hoffmann (1958). Dr. Alonso Zarazaga, an Apion taxonomist from Malaga, Spain, indicated to Drs. Whitehead and Clement that there is considerable taxonomic confusion in this taxa so the present subgeneric makeup is subject to change.

Table 1. Known host plant records for *Apion* weevils in the subgenus *Ceratapion* Schilsky, 1906.

Species and distribution	Host Plants
1) <i>A. carduorum</i> Kirby, 1808 Europe including France, Algeria, Syria, Greece Asia Note: Hoffmann recognizes two subspecies: <i>galactites</i> Wencher 1858 and <i>damryi</i> Desbr., 1893	<u>Centaurea solstitialis</u> <u>C. napifolia</u> <u>Cynara scolymus</u> <u>C. cardunculus</u> <u>Carduus acanthoides</u> <u>C. nutans</u> <u>C. pycnocephalus</u> <u>C. personatus</u> <u>Cirsium arvense</u> <u>C. oleraceum</u> <u>C. palustre</u> <u>C. lanceolatum</u> <u>Lappa communis</u> <u>Silybum marianum</u>
2) <i>A. armatum</i> Gestacker, 1854 Belgium, Denmark, Germany, Poland, Austria, France, Italy, Romania, Switzerland.	<u>Carlina vulgaris</u> <u>Echinops nitro</u> <u>Centaurea aspera</u> <u>C. jacea</u> <u>C. nemoralis</u> (=nigra)
3) <i>A. cylindricolle</i> Gyll., 1839 Romania, Russia, Turkey, France.	<u>Xeranthemum cylindraceum</u>
4) <i>A. fallaciosum</i> Besbr., 1892 France, Spain, Italy, Algeria.	<u>Xeranthemum inspertum</u>
5) <i>A. scalptum</i> Rey, 1859 France, Spain, Italy, Sicily, Sardinia, Yugoslavia, Algeria, Turkey.	<u>Centaurea solstitialis</u> <u>Cirsium anglicum</u> <u>C. vulgare</u> <u>C. lanceolatum</u> <u>Carthamus lanatus</u> <u>Carthamus</u> sp.
6) <i>A. alliariae</i> Herbst, 1792. France, Italy, Sicily, Greece, Algeria, Morocco.	<u>Carduus pycnocephalus</u> <u>C. tenuifloris</u> <u>Centaurea solstitialis</u> <u>Onopordum tauricum</u> "Intercepted with barley heads in Turkey" (White- head, pers. comm.).
7) <i>A. penetrans</i> Germar, 1817. France, Germany, Austria, Belgium, Russia, Italy (Liguria), Yugoslavia, Morocco.	<u>Centaurea jacea</u> <u>C. nigra</u> <u>C. cyaneus</u> <u>C. paniculata</u> <u>C. stoebe</u> (=C. <u>maculosa</u> ) <u>C. cyanus</u> <u>C. nemoralis</u> (=C. <u>nigra</u> ) <u>C. scabiosa</u>
8) <i>A. onopordi</i> Kirby, 1802 Europe including France, Asia Minor, Algeria.	<u>Centaurea nigra</u> <u>C. paniculata</u> <u>C. calcitrapa</u> <u>C. scabiosa</u> <u>C. amara</u> <u>C. amara</u> v. <u>Duboisii</u> <u>C. collina</u> <u>C. solstitialis</u> <u>C. stoebe</u> (= <u>maculosa</u> ) <u>C. jacea</u> <u>C. nemoralis</u> <u>Cnicus benedictus</u> <u>Onopordum illyricum</u> <u>O. acanthium</u> <u>Cirsium lanceolatum</u> <u>C. oleraceum</u> <u>C. rivulare</u> <u>C. canum</u> <u>C. arvense</u> <u>C. vulgare</u> <u>Carduus nutans</u> <u>C. crispus</u>
9) <i>A. austriacum</i> ? Note: Hoffmann lists this as a subspecies of <i>armatum</i> .	<u>Centaurea scabiosa</u>

Weevils in the subgenera Diplapion and Ceratapion are closely related. Apion (Diplapion) detritum Rey is recorded from C. solstitialis, Arctium minor, and Silybum marianum. According to Dr. D. Whitehead (pers. comm.) the small subgenus Omphalapion is the only other European group of Apion likely to be associated with Compositae.

## Objective 2

A number (n = 127) of adult Apion were submitted to the Systematic Entomology Laboratory, USDA, Beltsville, Maryland for identification. Except for one adult collected while sweeping a field of YST near Noci (Puglia region of Italy), all of the Italian specimens were reared from the crowns or roots of YST or Centaurea nicaeensis (identity of this plant was confirmed by Prof. Arrigoni, Istituto di Botanica, Universita' di Firenze). Dr. D. Whitehead "tentatively" identified the Apion reared from YST collected at five sites in Italy as A. alliariae and A. onopordi. The field collected adult was identified as A. alliariae, and the specimens reared from C. nicaeensis as "near" A. orientale Gerst.. All of the specimens reared from YST in Greece and Turkey were identified as A. alliariae

The specimens collected as feeding or resting adults on YST in Greece were identified as A. carduorum, a known pest of artichoke, and A. alliariae, A. onopordi, and A. scalptum. All of the adults collected by Drs. R. Sobhian and L. Andres on Carthamus lanatus and Carthamus sp. in Greece were identified as A. scalptum. One Apion (species unknown but not Ceratapion) was collected on YST in Turkey but its association with this plant was called "spurious" by Dr. Whitehead.

Perhaps we are dealing with one or two species in the rosettes but this needs to be confirmed by another Apion specialist. Dr. Whitehead

suggested that we try and get outside taxonomic opinions, and steps have been undertaken to do this.

#### Objectives 3 and 4

The three study sites near Rome and the two in southern Italy which yielded Apion larvae were visited at irregular intervals from March 4 to October 5.

The first weevil larvae ( $n = 2$ ) were found in a sample of 17 YST rosettes collected April 11 at a site (site 1) north of Rome. Yellow starthistle plants at this site were concentrated in a narrow strip (2 m wide) along a dirt road near Via Cassia.

A trip was made on April 27 to the Campania and Puglia regions of southern Italy where stops were made near Benevento (site 4) and at Castel del Monte (site 5). Eight of 43 rosettes from site 4 (narrow strip of land along Highway 88 near Benevento) contained weevil larvae. These larvae had not yet entered the roots; they were feeding at the point where the leaves radiate out from the crown. Weevil larvae were found in the crowns and roots of 21 of 86 randomly selected YST rosettes removed from site 5, which was a large (ca. 8 acres) field with a sizeable YST infestation. Another series of rosettes ( $n = 125$ ) was dug up from this plot and each plant was dissected to record the mean ( $\pm$  SD) number of larvae per infested plant. The number recorded was  $1.27 \pm 0.46$ . An additional 90 plants were removed from the  $400\text{m}^2$  plot and taken to Rome where they were transplanted into a garden at the Laboratory. The objective was to try and establish a resident population of Apion in the Laboratory garden.

Weevil larvae were recovered from YST rosettes collected on May 3 at site 1 and 2. Site 2 is about 30 km north of Rome near Lake Bracciano and is where P. Dunn first observed extensive damage to YST and attributed it to

Apion sp. Five of 15 rosettes from site 1 contained larvae while 5 of 9 from site 2 harbored larvae. A sample (n = 11) of rosettes collected on May 18 at site 1 yielded Apion larvae in 5 plants, pupae in 2 plants, and a teneral adult in one plant. Plants collected April 27 at Castel del Monte and subsequently held in a controlled temperature room ( $22 \pm 5^{\circ}\text{C}$ ; 16 h light) at the Rome Laboratory yielded 13 adults on May 24. A May 26 plant sample (n = 58) from a field along the Appia Antica near Rome (site 3) yielded Apion larvae in 5 plants and pupae in 2 plants.

Five Apion adults were observed feeding on May 30 on some of the Castel del Monte plants in the Rome garden. Adults were consistently observed on these plants through late-June. Two adult Apion were extracted from the upper root section of a YST plant removed from site 1 on June 14.

A goodly number of adults were seen feeding on mature YST plants at site 4 (Benevento) on June 27 and 15 were collected and subsequently held indoors in plastic containers with bouquets of YST leaves and buds. Bouquets were changed two or three times per week. These weevils all died by late-September as did 33 other Apion adults that were held in a similar manner after they were reared out from YST collected at the other study sites. It was hoped these adults would eventually supply eggs for a host specificity test but this was not accomplished so other methods and approaches will be tried in 1984.

One adult Apion was collected on September 30 while feeding on a "diseased" YST plant in a field near Noci (Puglia). It was apparent that this plant was suffering from some malady, because it was still green in contrast to most of the YST plants which by late-September had all but dried up.

None of the YST rosettes harboring Apion larvae showed any visible signs of stress but this aspect needs further study before one can judge if

the insect is having any impact on the host plant.

From the information presented above it is possible to sketch a partial life history of the YST Apion. Eggs are probably laid during a 2-3 month period (March - May) Larvae could develop from late-March to early-May or even later and pupation could occur as early as the first or second decade of May. New adults could appear in early-or mid-May. More study is certainly required before we can piece together an accurate picture of the life history of the rosette and root inhabiting Apion. It will help if in 1984 we can determine the fate of the adults after late-June and pinpoint mating and oviposition periods. As a result of a February 6, 1984 field trip to sites 4 and 5 it was discovered that eggs had not yet been laid in rosettes (diameter of 10 rosettes was  $9.85 \pm 2.67$  cm at site 4; spread of 30 rosettes was  $14.97 \pm 4.47$  cm at site 5).

Some additional information on the level of the Apion infestation at Castel del Monte was obtained on June 27. Ten one m<sup>2</sup> plots were established (ca. 5 m between plots) along a transect in this field and all of the YST plants per plot were removed and dissected to record the infestation level. Larval and pupal development was mostly completed by this date. We felt it was possible to estimate the number of larvae that each plant had harbored because previous dissections of about 130 infested plants had provided an indication of the type and extent of damage that a set number of larvae could cause to a root. This approach was appropriate for determining the relative number of larvae that developed in infested plants. Table 2 shows that at least 40.7% (n = 81) of the plants were attacked by Apion. Another point worth stressing is that a plant usually supported a low number (1-3) of larvae.

Cyphocleonus sp.

Two specimens of a rather large weevil were reared from the roots of

YST that were removed from site 1 on June 14. These may be Cyphocleonus morbillosus F.. This specimens were returned over to Gaetano Campobasso since he is studying the biocontrol potential of this species. Four other large weevil larvae (presumably Cyphocleonus) were recovered from this site but they died before completing development.

#### Yellow Starthistle Garden

The plot design for this YST garden was a 6 x 6 Latin square with one plant per block. The six treatments were: YST from Italy (Brindisi) which served as the control; YST from Spain; YST from Concord, California ; YST from Tehama County, California; YST from Walla Walla, Washington; and YST from Yakima, Washington. A distance of 1 m separated plants and the entire plot was surrounded by several hundred Italian YST plants. The test plants were started from seed on January 31, 1983 in a greenhouse and transplanted to the field plot as rosettes on March 25.

It is hoped that this plot will provide an indication of the ability of the Italian biotype of Urophora sirunaseva (Hering) to find, attack and develop in the heads of different ecotypes of YST. This field experiment, and others that are planned for Italy and Greece in 1984, will allow us to quantitatively assess the host utilization patterns of the insect guild associated with YST seed heads.

Mature seed heads per plant were removed once a week from July 8 to September 20. These were placed in cardboard containers fitted with organdy cloth covers and held in a laboratory so emerging insects could be collected. A full account of the results will be prepared after this planned two year study is completed. However, it should be mentioned that some tephritid flies emerged in the fall in the containers and these were identified as: 1) Urophora quadrifasciata (Meigen) (Diptera: Tephritidae) (det. by Dr. Peterson,

Research Entomologist, USDA Systematic Entomology Laboratory). These were reared from heads of Italian, Walla Walla and Yakima, Washington plants, and 2) Urophora sirunaseva (Hering) (Diptera: Tephritidae) (det. by Dr. Peterson). One specimen was reared from an Italian plant.

The number ( $\bar{x} \pm SD$ ) of seed heads produced by each ecotype is shown in Table 3. The results reveal considerable variability within each ecotype.

#### Additional Natural Enemies

Our surveys turned up some other natural enemies which may or may not be potential biocontrol agents of YST. Some of these species have been singled out by previous biocontrol researchers as having biocontrol potential but we do not know how much "follow-up" work was done. This aspect will be examined as time permits. These natural enemies are listed below.

Natural Enemy	Stage of Plant Attacked	Source of Material	Source of Identification.
1. <u>Eriophyid</u> sp.	Flower head	YST collected July 20 near Noci, Italy.	-
2. <u>Eublemma parva</u> (Hubner) (Lepidoptera: Noctuidae)	Flower head	YST plants in Rome garden	Dr. Poole, USDA Systematic Entomology Laboratory.
3. Unidentified tortricid moth	Larvae are leaf rollers in rosettes.	From potted YST at Rome Laboratory	-
4. <u>Tebenna</u> sp. <u>prob. micalis</u> (Mann) (Lepidoptera: Choreutidae)	Larvae are assumed to feed on foliage	Yst plants in Rome garden	Dr. Hodges, USDA Systematic Entomology Laboratory.
5. <u>Metzneria aprilella</u> (Herrich-Schaffer) (Lepidoptera: Galechiidae)	Flower head	YST from Castel del Monte	Dr. Hodges, USDA Systematic Entomology Laboratory

Dunn and Clement made a July 20-21 trip to Puglia to try and collect U. sirunaseva adults for D. Maddox, USDA Albany Laboratory. About 40 adults were collected but they did not survive the return trip to Rome. What was discovered in 4 fields during this trip were YST plants suffering from some malady. Dott. Cristofaro found plants showing the same stunted growth symptoms at a site north of Rome in early summer. A "mycoplasma-like" organism might be responsible for this disorder. These plants do not produce many viable seeds. Attempts will be made in 1984 to learn more about this disease and the extent to which it occurs in central and southern Italy.

#### Survey of Closely Related Plants

Only one other Centaurea species was surveyed and this was the biennial plant C. nicaensis (determined by Prof. Arrigoni, Istituto di Botanica, Universita' di Firenze).

A survey along highway #16 between San Severo and Serracapriola resulted in the discovery of several hundred of these plants in embankments and bordering fields.

Thirteen plants were collected on March 16 at a site 700 m NE of road marker # 39 after S. Paolo di Civitate. These plants were potted and placed in a controlled temperature-light room ( $22 \pm 5^{\circ}\text{C}$ ; 16 h light) where they were caged so any emerging insects could be collected. Three species of insects emerged:

- 1) Apion sp. nr. orientale Gerstaecker (Coleoptera: Curculionidae).

Identified provisionally by Dr. Don Whitehead, Research Entomologist USDA Systematic Entomology Laboratory; 2) An unidentified tortricid moth reared from leaf-rolling larvae on pre-bolting stage plants;

- 3) Pterolonche inspersa Standinger (Lepidoptera: Pterolonchidae).

Identified by Dr. R. Hodges, Research Entomologist, USDA Systematic

Entomology Laboratory. Larvae developed in the roots. This insect is a prime candidate for the biological control of diffuse knapweed.

A fourth species, Urophora quadrifasciata (Meigen) (Diptera: Tephritidae) (det. by Dr. Peterson, Research Entomologist, USDA Systematic Laboratory) was collected as adults in mid-summer. The flies were captured on mature flower heads of C. nicaeensis.

Table 2. Number of Apion larvae that completed their development in each infested Centaurea solstitialis plant collected at Castel del Monte, Italy, June 27, 1983.

Plot No. <sup>1/</sup>	No.Plants/Plot	Infestation Level			
		1-2	2-3	3-4	4-5
		larvae	larvae	larvae	larvae
1	8	1 <sup>2/</sup>	-	-	-
2	4	1	2	-	-
3	5	2	-	-	-
4	11	3	1	1	-
5	5	1	-	1	-
6	7	4	1	-	-
7	10	4	1	-	1
8	7	2	-	-	-
9	6	-	1	-	-
10	18	4	2	-	-

<sup>1/</sup> Plots were 1m<sup>2</sup> and separated by ca. 5 m.

<sup>2/</sup> Number of plants.

Table 3. The mean ( $\pm$  SD) number of seed heads produced by each of the six Centaurea solstitialis ecotypes in the Latin square plot, Rome, 1983.

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Ecotype	Mean ( $\pm$ SD) No. Seed Heads	Minimum and Maximum number of seedheads per plant.
<hr/>		
Italy (control)	363.0 $\pm$ 350.8 (5) <sup>1/</sup>	34 - 773
Spain	152.5 $\pm$ 80.4 (6)	31 - 261
Concord, Calif.	208.0 $\pm$ 142.0 (4)	35 - 334
Tehama, Calif.	237.7 $\pm$ 138.3 (6)	80 - 392
Walla Walla, Wash.	108.2 $\pm$ 115.6 (6)	25 - 303
Yakima, Wash.	278.7 $\pm$ 180.2 (6)	73 - 593

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<sup>1/</sup> (n); One Italian plant and two Concord plants died before they produced flower heads.

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CENTAUREA SOLSTITIALIS

P. Dunn, G. Campobasso, F. Murano (part-time)

Bangasternus orientalis (Capiomont) (Coleoptera: Curculionidae)

Based on the positive results obtained on 1982 (see Annual Report) we decided for 1983 to continue the screening of the seed feeding weevil B. orientalis (C.). Following the plant list developed by D. Maddox (Albany, CA.) and later modified by P. Dunn (Rome, Italy) 31 plant species have been tested with the weevil. Live adults of B. orientalis were collected in Greece, and the plants used in the tests were grown at the Rome Laboratory. In order to ascertain the host range of the weevil "no choice" oviposition tests and 1st instar larval survival trials were made at the Rome Laboratory during May, June, and July 1983.

1983 Work Plan Followed

Collection trip: With the cooperation of Dr. R. Sobhian; and Dr. L. Andres six hundred (600) Bangasternus orientalis adults were collected in Greece, near Thessaloniki and Kazmi, in May. At the Thessaloniki Laboratory these weevils were separated by sex and kept in the refrigerator (10°C) in order to slow down their activity. On May 20 these adults were hand carried to the Rome Laboratory and experiments were started on May 26. 120 eggs were collected from 70 plants of Centaurea solstitialis near Thermi (Thessaloniki) in order to determine the presence of egg parasite and the percentage of egg parasitism, if any. These eggs were kept in eclosion cups (small plastic cups with a moist plaster of paris substrate) until eclosion.

Experiments: Single plant (No choice oviposition test): The plant selected for this test are listed in table 1. The experiment was carried out in the greenhouse with the temperature ranging between 20-35°C, the RH

ranging from 30 to 90% (outside the cages) and the photoperiod ca 16h. The insects were confined to plants in pots, each covered by a transparent plastic cylinder (diameter 20 cm; height 70 cm) with four holes (10 cm diameter) covered with organdy on the sides of each cylinder to permit air circulation. Each tube was capped with organdy cloth held in place by a large rubber band. On 26 May, 5 replicates of each plant were infested with 2<sup>00</sup> and 200 B. orientalis adults. On July 18, after most of the adults had died, all the test plants were examined, and total oviposition recorded. The results of this trial are summarized in Table 1.

First instar larval survival: The same plant species used in the preceding test were used in this experiment. The test was conducted in the garden from June 10 - July 10 under ambient temperature and relative humidity. One bud on each one of the 10 replicate test plants was infested with 2 fertile eggs (total of 20 eggs per test plant). A fine brush was used to transfer the fertile B. orientalis eggs, which were placed between the bracts of the immature buds of the test plants. The buds were marked in order to facilitate re-examination and allow us to record the number of eggs hatching. All the eggs hatched. The test lasted until July 11-12 when the infested flower heads were dissected under a stereomicroscope recording the number of living larvae found. Results are presented in Table 2.

Adult's Oviposition Site Preference: During oviposition time of B. orientalis, the natural host (Centaurea solstitialis) may have as many as six different floral bud stages present at one time. In order to establish which bud stage of the host plant is preferred by B. orientalis an experiment was conducted from May 25 until June 2, 1983. Following the figure prepared by D. Maddox we were able to discriminate the various bud stages that C. solstitialis could have during May-June.

Yellow starthistle in pots were used for this test, and 1 pair of insects were caged on each test plant using the same plastic cylinders as in the preceding test. Ten plants (replicates) with all the bud stages present were used in this trial which was carried out in a greenhouse at temperatures ranging from 20-35°C and an RH outside the cages of 30-90% and 16 hours L/D photophase. The results are shown in Table 3.

Egg Mortality Factor: In order to have a better understanding of the reason for the high naturally occurring egg mortality in the field. Three different parasites, 2 of which were identified only to family as Mymarids and a Trichogrammatid Pterondrophysalis levantina were the causes of the high egg mortality. Based on our sample of 120 field collected eggs only 4 larvae of B. orientalis were not parasitized. Other biotic factors which could attack the egg stage of the weevil were not investigated.

#### Results and discussions

The results so far obtained from the two experiments (oviposition, and larval survival test) have shown that B. orientalis has a restricted host plant range. Oviposition occurred on different biotypes of Centaurea solstitialis, C. diffusa, C. jacea, and Onopordium acanthium, but the first instar larvae were able to survive only on Centaurea solstitialis. No trace of larval feeding was found on any plant of economic importance. At present, based on the positive results obtained we feel that continued screening of this weevil is justified.

1984 Work Plan

The following plants will be tested with B. orientalis (Capiomont).

- 1) Centaurea solstitialis (control Greece)
- 2) C. paniculata
- 3) C. maculosa
- 4) C. montana
- 5) C. americana
- 6) C. calcitrapa
- 7) C. aspera
- 8) Carduus crispus
- 9) C. personatus
- 10) C. thoermeri (US)
- 11) C. nutans (US)
- 12) C. acanthoides (US)
- 13) Cynara scolymus (US)
- 14) Lattuce great lake
- 15) Onopordum acanthium
- 16) Echinops sphaerocephalus
- 17) Carlina vulgaris
- 18) Saussurea alpina
- 19) Arctium minus
- 20) Cnicus benedictus
- 21) Artemisia vulgaris
- 22) Solidago canadensis
- 23) Erigeron annuus
- 24) Scabiosa atropurpurea
- 25) Sonchus oleraceus

- 26) Viola bertolini
- 27) Tanacetum vulgare
- 28) Euphorbia lathyris

Insect collection: Collect Bangasternus orientalis (C.) in Greece during May and send to Rome for continuing the host specificity tests.

Study larval instars (how many instars, time between molts).

To establish a colony for oogenesis test for 1985 studies. Adult starvation trials to see which plants should be in oogenesis trials.

The genus Bangasternus will be revised by an Italian Curculionidae specialist (Dr. Enzo Colonnelli, University of Rome) with the cooperation of the Director of the Rome Laboratory and his assistant G. Campobasso.

Table 1. Oviposition on single plant (Plants dissected after 45 days)

Replicate No.	1	2	3	4	5	Total eggs
<u>Plants tested</u>						
<u>Centaurea solstitialis</u> (Greece) control	120	180	100	150	160	710
<u>Centaurea solstitialis</u> (Salerno)	4	20	60	80	120	320
<u>Centaurea solstitialis</u> (Bracciano)	130	105	70	20	60	385
<u>Centaurea solstitialis</u> (Noci)	82	100	82	150	75	489
<u>Centaurea solstitialis</u> var. Schowii	0	35	45	80	80	240
<u>Centaurea diffusa</u>	15	0	0	15	0	0
<u>Centaurea jacea</u>	0	70	3	0	73	0
<u>Centaurea calcitrapa</u>	0	0	0	0	0	0
<u>Centaurea scabiosa</u>	0	0	0	0	0	0
<u>Carduus nutans</u>	0	0	0	0	0	0
<u>Carduus pycnocephalus</u>	0	0	0	0	0	0
<u>Carduus tenuiflorus</u>	0	0	0	0	0	0
<u>Silybum marianum</u>	0	0	0	0	0	0
<u>Cynara scolimus</u>	0	0	0	0	0	0
<u>Onopordum acanthium</u>	7	0	0	0	7	0
<u>Senecio jacobaea</u>	0	0	0	0	0	0
<u>Tagetes erectus</u>	0	0	0	0	0	0
<u>Zinnia elegans</u>	0	0	0	0	0	0
<u>Rudbeckia hirta</u>	0	0	0	0	0	0
<u>Achillea millefolium</u>	0	0	0	0	0	0
<u>Tanacetum vulgare</u>	0	0	0	0	0	0
<u>Anthemis tinctoria</u>	0	0	0	0	0	0
<u>Cirsium lanceolatum</u>	0	0	0	0	0	0
Lattuce Great Lakes	0	0	0	0	0	0
<u>Leontodum crispus</u>	0	0	0	0	0	0
<u>Calendula officinalis</u>	0	0	0	0	0	0
<u>Silene vulgaris</u>	0	0	0	0	0	0
<u>Ranunculus auricanus</u>	0	0	0	0	0	0
<u>Linaria dalmatica</u>	0	0	0	0	0	0
<u>Papaver somniferum</u>	0	0	0	0	0	0
<u>Taraxacum officinale</u>	0	0	0	0	0	0

Table 2. First instar larval survival trial (Plants dissected 30 days after infestation).

Replicate No.	1	2	3	4	5	6	7	8	9	10	
Plant Tested	No. larvae alive at dissection										Total larvae alive
<u>Centaurea solstitialis</u> (Greece) 1st control	2	1	1	1	1	1	1	1	0	1	10
<u>Centaurea solstitialis</u> (Greece) 2nd control	1	1	1	2	1	1	2	2	3	1	15
<u>Centaurea solstitialis</u> (Salerno)	0	0	1	0	1	1	1	1	1	1	7
<u>Centaurea solstitialis</u> (Bracciano)	1	1	1	1	1	1	1	1	1	1	10
<u>Centaurea solstitialis</u> (Noci)	1	1	0	1	1	1	1	1	1	0	8
<u>Centaurea solstitialis</u> var. Schowii	0	0	1	0	0	0	0	1	1	0	3
<u>Centaurea diffusa</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Centaurea jacea</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Centaurea calcitrapa</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Centaurea scabiosa</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Carduus nutans</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Carduus pycnocephalus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Carduus tenuiflorus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Silybum marianum</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Cynara scolymus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Onopordum acanthium</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Senecio jacobaea</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Tagetes erectus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Zinnia elegans</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Rudbeckia hirta</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Achillea millefolium</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Tanacetum vulgare</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Anthemis tinctoria</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Cirsium lanceolatum</u>	0	0	0	0	0	0	0	0	0	0	0
Lattuce Great Lakes	0	0	0	0	0	0	0	0	0	0	0
<u>Leontodon crispus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Calendula officinalis</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Silene vulgaris</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Ranunculus auricomus</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Linaria dalmatica</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Papaver somniferum</u>	0	0	0	0	0	0	0	0	0	0	0
<u>Taraxacum officinale</u>	0	0	0	0	0	0	0	0	0	0	0

\* Two fertile eggs placed on each plant replicate - four fertile eggs placed on Centaurea solstitialis (Greece) 2nd control.

Table 3. *B. orientalis* test to determine preference of yellowstar thistle budsize for oviposition site.

Rep #	Stage Bu 1			Stage Bu 2			Stage Bu 3			Stage Bu 4			Stage F-1			Stage F-2		
	# buds	# Floral buds infested	# Eggs found	# buds	# Floral buds infested	# Eggs found	# buds	# Floral buds infested	# Eggs found	# buds	# Floral buds infested	# Eggs found	# buds	# Floral buds infested	# Eggs found	# buds	# Floral buds infested	# Eggs found
1	19	10	11	1	1	2	1	1	1	1	1	1	-	-	-	-	-	-
2	28	22	34	6	6	23	1	1	10	-	-	-	-	-	-	-	-	-
3	61	24	57	4	4	18	-	-	-	-	-	-	-	-	-	-	-	-
4	22	10	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	33	19	42	4	4	18	1	1	5	-	-	-	-	-	-	-	-	-
6	50	49	180	-	-	-	1	1	10	-	-	-	-	-	-	-	-	-
7	18	15	60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	23	18	48	2	2	6	1	1	3	-	-	-	-	-	-	-	-	-
9	37	33	92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	24	22	95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	322	222	479	17	17	69	5	5	29	-	-	-	-	-	-	-	-	-

see figures of floral buds in the back sheet.

CYPHOCLEONUS MORBILLOSUS (Fabricius) (Coleoptera: Curculionidae)

P. Dunn; G. Campobasso, F. Murano (part-time)

The decision to work with the root feeding weevil C. morbillosus was prompted by the fact that there are no root feeding candidate natural enemies of yellow starthistle. In 1965 Dr. H. Zwolfer, CIBC Delemont Laboratory, conducted some preliminary feeding tests using two field collected adults of C. morbillosus (F.). These two adults were caged with potted plants of Carthamus tinctorius and Cynara scolymus. The Carthamus was not touched, during the trial but the small artichoke plant was destroyed after 10 days of adult feeding. Since the adult in our preliminary experiments conducted in laboratory during April-May didn't accept Cynara scolymus, we felt that we should continue with the screening of this weevil.

1983 Work Program

Literature Search: Cyphocleonus morbillosus (F.) is a curculionid belonging to the subfamily Cleoninae. This species was first described by Fabricius in 1793, and according to Motschulsky (1860) there are 12 species in the genus Cyphocleonus. (See table #1 for species, distribution and host plants). Della Beffa (1961), and Balachowsky (1963) provided no literature records of C. morbillosus as a pest of cultivated plants.

Survey trips: In order to find populations of C. morbillosus (F.) a survey trip was made to Southern Italy during April 1983. The search, was concentrated in the regions of Campania where there was a previous collection record by L. Andres and Puglia because the target plant is widely distributed there. Plants of Centaurea solstitialis from both regions were inspected and dissected. No larvae of C. morbillosus were found during April in the roots of C. solstitialis rosettes. However, a single female C. morbillosus adult

was found feeding on the rosette crown of C. solstitialis in a small undisturbed area (ca. 50 square meters) in Campania, near Salerno. The weevil was brought back to the laboratory where preliminary tests were conducted.

#### Experiments:

Oviposition on host plant: In order to determine if this single field-collected female of C. morbillosus was gravid, it was caged with a potted yellow starthistle plant in a clear plastic cylinder cage (diameter 20 cm; height 70 cm) with four organdy covered holes (diameter 10 cm) on the sides of the cylinder (to permit air circulation) and capped with organdy cloth held in place by a large rubber band. Every two days, the host plant was changed and the plant which had been exposed to the weevil was carefully checked, recording the oviposition sites, number of eggs, and any damage done to the host. The experiment was conducted in the laboratory garden under natural climatic conditions and lasted from April 22 until June 20.

Oviposition-Feeding Choice Tests: For this preliminary test the same female C. morbillosus was used. The test was conducted in the laboratory garden under natural conditions (June 20-27). Two important crop plants, (Cynara scolymus and Lactuca sativa (US seeds)) plus the control Centaurea solstitialis (Salerno) were transplanted together in a 35 cm diameter pot, and caged under a transparent cylinder (diameter 30 cm, height 60 cm). Every day the plants were checked, recording number of eggs deposited and feeding damage on the test plants. Results are summarized on Table 2 B.

First Instar Larval Survival Test: In order to find if the first instar larvae of C. morbillosus were able to accept American biotype of C. solstitialis and the crop Cynara scolymus as hosts, a preliminary test was conducted in the laboratory under natural conditions during the months of May-June. The following potted test plants were selected: Centaurea

solstitialis (Salerno control), C. solstitialis (Yakima WA), C. solstitialis (Goldendale WA), C. solstitialis (Tehama CA), and Cynara scolymus (California). Using a fine camel's hair brush each test plant was infested with one fertile egg (head capsule visible) . We marked the position of each egg placed on each test plant in order to find them easily and record if they hatched or not. The test was replicated 5 times, and lasted from May 20 until July 11, when the experiment was stopped. All the plants were dissected under a stereo-microscope and the surviving larvae were collected counted, and stored in ethyl alcohol. The results are presented in Table 2A.

Cyphocleonus morbillosus Laboratory Colony: In order to establish a laboratory colony of C. morbillosus, 50 plants of Centaurea solstitialis from Salerno were each infested with one first instar larva. Most of these infested plants were kept in the garden until the end of June but on June 15 some (n = 5) were dissected under a stereo-microscope in order to follow the larval development of the weevil.

This examination disclosed that all five plants had been attacked by a Diptera whose larvae (pest of cultivated plants Delia sp.), destroyed the root system especially the crown where the C. morbillosus larva starts to feed. On June 30 the remaining plants (n = 45) were dissected and no living larvae of C. morbillosus were found because of the fly damage to all of the plants.

The fly which caused this damage is poliphagous so it cannot be considered for biological control. (Insect sent for identification).

#### Results and Discussions

The results of these preliminary screening trials are very encouraging. The two crop plants Cynara scolymus and Lactuca sativa (Great Lakes) were not attacked. Also, the first instar larvae of C. morbillosus accepted the American biotypes of Centaurea solstitialis as host plant. In

the oviposition test, the female of C. morbillosus laid about 170 eggs, of which 150 were fertile. The eggs were laid around the crown in groups of 3-4 and well fixed to the host. The maximum number of eggs found on any one plant was 8 and the minimum number was 1. The length of the oviposition period was estimated to be 48 days. The results of this initial test indicate that the insect cannot develop on artichoke or lettuce and that it accepts the US yellow star varieties, so a more complete screening, using larger numbers of insects and a larger list of test plants is in order.

1984 Work Plan:

1. At the beginning of May we plan to make a collection trip in Salerno and as many as possible adults of Cyphocleonus morbillosus will be collected in order to conduct more complete host specificity tests and biological studies.

2. The following plants were selected for the 1984 tests:

- 1) Centaurea solstitialis (Salerno, control)
- 2) Centaurea solstitialis (Yakima, US)
- 3) Centaurea solstitialis (Goldendale, US)
- 4) Centaurea solstitialis (Walla Walla, US)
- 5) Centaurea solstitialis (Contro costa, US)
- 6) C. diffusa (host of Cyphocleonus achates)  
personal record
- 7) C. paniculata (host of C. tigrinus) Hoffmann  
record
- 8) Achillea millefolium (host of C. tigrinus) Hoffmann  
record
- 9) Andryala integrifolia (host of C. tigrinus) Hoffmann  
record

- 10) Artemisia vulgaris (host of C. tigrinus) Hoffmann  
record
- 11) A. absinthium (host of C. tigrinus) Hoffmann  
record
- 12) Leucanthemum vulgare (host of C. trisulcatus)  
Hoffmann record
- 13) Carduus thoermeri
- 14) C. acanthoides
- 15) Beta vulgaris var. saccariphera
- 16) Cirsium undulatum (US)
- 17) C. occidentale (US)
- 18) Cynara scolymus (US)

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Table 1. Biological information on Cyphocleonus spp. Host records and Distribution.

<u>Species of Cyphocleonus</u>	<u>Known Geographic Distribution</u>	<u>Recorded Host Plants</u>
<u>cenchus</u> (Pallas, 1781)	Southern Russia, Caucasus, Turkestan, Iran, and Kazakhstan	Unknown
<u>sparaus</u> (Gyllenhal, 1834)	Corsica, Italy, Morocco, Iran and Mesopotamia	unknown
<u>tigrinus</u> (Panzer, 1781)	Southern Central Europe, Caucasus, Iran	<u>Centaurea paniculata</u> L.
<u>(marmoratus-Fabricius, 1793)</u>	China and Kazakhstan	<u>Andryala integrifolia</u>
<u>achates</u> (Fahraeus, 1842)	Hungaria, Syria, Eastern Europe, and Greece	<u>Centaurea diffusa</u> Lam.
<u>(achatesides, Chev. 1873)</u>		
<u>morbillosus</u> (Fabricius 1793)	Asia, Italy, France, Morocco and Algeria	<u>Centaurea solstitialis</u> L.
<u>(fibox, Boheman, 1842)</u>		<u>Centaurea paniculata</u> L.
<u>(hedenbargi, Fahraeus, 1842)</u>		
<u>(testatus, Gyllenhal, 1834)</u>		
<u>(gallicus, Hoffmann, 1950)</u>		
<u>armitagaei</u> (Wollaston, 1864)	Tenerife	unknown
<u>lejeunei</u> (Fairmaire, 1866)	Algeria	unknown
<u>(exanthematicus, Frm., 1866)</u>		
<u>trisulcatus</u> (Herbst, 1795)	Southern Central Europe	<u>Leucanthemum vulgare</u> L.
<u>altaicus</u> (Gebler, 1830)	Southern Russia and Syberia	unknown
<u>adumbratus</u> (Gyllenhal, 1834)	Southern Russia, Kazakhstan, Syberia and Hungaria.	unknown
<u>(occultus, Fahroeus, 1842)</u>		
<u>samaritanus</u> (Reiche, 1857)	Palestina	unknown
<u>sventaniusi</u> (Roudier, 1956)	Gran Canaria	unknown

Table 2 A. First instar larval survival trial (Plants dissected 30 days after infestation)

Replicate No. Plants tested	No. larvae alive at dissection					Total larvae alive
	1	2	3	4	5	
<u>Centaurea solstitialis</u> (Salerno)	1	1	1	1	1	5
<u>Centaurea solstitialis</u> (Yakima WA)	1	0	1	0	1	4
<u>Centaurea solstitialis</u> (Golden dale WA)	1	1	1	1	0	4
<u>Centaurea solstitialis</u> (Tehama CA)	1	1	0	1	1	4
<u>Cynara scolymus</u> (USA)	0	0	0	0	0	0

1/ One fertile egg placed on each plant replicate.

Table 2 B. Oviposition Choice and adult feeding preference test (Experiment lasted 7 days).

Test Plant	Total eggs recovered	Total feeding <sup>1/</sup> damage mm <sup>2</sup>
<u>Centaurea solstitialis</u> (Salerno control)	14	450
<u>Cynara scolymus</u> (USA)	0	0
<u>Lactuca sativa</u> Great lakes (USA)	0	0

1/ Feeding damage caused by one ♀ C. morbillosus adult.

075



EUSTENOPUS VILLOSUS

R. Sobhian

Thessaloniki, Greece

Material and Methods

The weevils used for the experiments were collected from Doirani and Thermi, Greece, the American varieties of yellow starthistle (YST) were from seeds provided by USDA Biological Control of Weeds Laboratory, Albany, California, and the American varieties of safflower seeds were provided by the USDA Biocontrol of Weeds Laboratory, Rome, Italy.

For oviposition tests, field collected adult weevils of E. villosus were caged in gauze bags on safflower and YST plants growing in the garden, in the laboratory oviposition they were caged with safflower and YST bouquets in glass cylinders (20 cm diameter and 20 cm high) covered with a piece of cardboard.

To determine host preference, seed heads of safflower , wild Carthamus sp. and YST collected from the field at Thermi were examined for the presence of Eustenopus. In order to determine the impact of the species on YST, the number of seeds in a sample of seed heads infested only with E villosus was counted and compared with uninfested seed heads When a seed head contained an Eustenopus cocoon, the seeds were counted, regardless of the content of the cocoon (ie., adult, and pupae or last instar larvae living or parasitized). Seed heads with evidence of oviposition were measured at the largest diameter and examined for Eustenopus infestation then the seed heads were segregated according to size. For a control the mean number of seeds in 10 uninfested seed heads in each size range was used.

### Biology

Eustenopus villosus has one generation per year, overwintering as an adult. In Thermi the search for adults emerging in the spring started in March and continued into May. The first adult was observed in the field on May 9. On June 3, after 10 hours search in Doirani, 70 adults were collected, all of them were collected as single individuals (no copulation or pairs observed). On June 21, the beetle was more common, 60 adults were collected in 6 hours of searching. Among these were only 5 pairs and the rest were collected as single individuals. On July 26, the adults were even more common and were generally found copulating on YST plants. Some of the adults collected on June 21 in Doirani and caged on YST at the University farm were still alive on July 28.

The first adult emerged on August 7, from YST seed heads collected in Thermi on July 13. The adults which were dissected from or emerged from seed heads in the laboratory on August and September were inactive and when kept in a transparent plastic container along with the residuals of dissected YST heads, they were observed to rest and hide on or among the plant material. In nature they probably overwinter in plant debris in the field.

The adults are quite harmful to the host plant. When feeding they make deep holes in very young flower buds damaging them severely, causing them to dry out. In captivity they also damage axillary buds in the same way, their long and thin rostrum being adapted for this kind of feeding. A large YST plant on which 60 adults were caged on June 3, showed heavy damage on June 12, all but 3 of the flower buds of the plant were partially or completely eaten while a control plant of about the same size had over 100 flower heads when the examination was made.

The <sup>00</sup> usually select floral bud stage BU4 (Maddox 1981) for

oviposition. They cut one hole in the bud, lay 1-2 oval yellowish eggs in it then cover it with a black-brown (perhaps fecal) material. The plant tissue below the hole in which the egg are laid turns to a soft jelly like material. This tissue change was observed 2 days after the oviposition and was also seen on buds offered as bouquets for oviposition. It is suspected that an enzyme is introduced to the bud in the course of oviposition to alter the substrate and make it acceptable for the first instar larvae. If this is true the enzyme might be a specific chemical that could be produced synthetically and used as a specific herbicide against YST.

Very often there were feeding holes without eggs found in the buds. Some of the holes are made by ♂♂ but it seems that the ♀♀ also make oviposition or feeding holes without laying in them. The holes are covered only if they oviposit in them.

At a temperature of 27-29°C and RH 60-70% the eggs hatch 3 days after oviposition. The larvae feed on the immature achenes thus preventing seed formation then pupate in flower heads in a loose cocoon. The adults emerge and leave the seed heads in August and Sept. Over 20,000 YST seed heads were dissected in October to collect Urophora sirunaseva galls, and despite evidence of Eustenopus damage to the seed heads normal U. sirunaseva galls were also found in the heads.

#### Host specificity and preference test

##### A - Laboratory tests:

Test I - YST bouquets of Greek and American origin, with flower buds all in the same stage were offered to Eustenopus for oviposition in glass cylinder cages. Each of the two replicates had 2 pair of weevils and the bouquets were replaced and examined daily for 3 days (June 28-30). Table I

shows the results of the experiment and that there seems to be a marked preference for the Greek strain of yellow starthistle.

Table I. Preference test with US and local YST

	Day	No.buds fed on	No.feeding holes	No.eggs	Total No.eggs
US-YST	1st	2	3	0	
	2nd	2	9	3	4
	3rd	3	6	1	
Local YST	1st	2	1	3	
	2nd	2	5	4	11
	3rd	3	8	4	

Test II - On June 30 the same 4 pairs of Eustenopus adults used for the above test with YST bouquets were caged with a safflower bouquet which had 3 buds in 3 different stages, to see if they would oviposit. The buds were replaced and examined the next day (July 1). No evidence of oviposition was found on the safflower buds but there were several feeding holes. The second safflower bouquet, placed in the test on July 1 was examined 3 days later (July 4) and had several feeding holes, but no eggs were found.

Test III - A larval feeding test was made with a small number of eggs and 1st instar larvae. On July 4, one egg and one 1st instar larva were transferred from YST buds to safflower buds and 3 1st instar larvae from YST to YST buds (one egg or larva/bud). On July 6 all the transferred larvae,

plus the one that hatched from the egg transferred to safflower were found feeding in their buds. On July 11 a final dissection was made and the 3 larvae placed on YST were still feeding in the buds, but the larvae in the safflower buds were dead.

II - Field experiment:

Test IV - Small branches of Greek and US yellow star and safflower each having 2-6 buds were caged with 5 adults each on June 27 and examined on July 4.

Several feeding holes and one egg were found on the US yellowstar branch, while the Greek yellowstar buds had 2 first instar larvae, one egg and several feeding holes. The safflower had only feeding holes and no eggs or larvae were found indicating that safflower is not acceptable for oviposition or that there were no females in the group confined with safflower.

In a second trial using Greek yellowstar and safflower plants, 2 pairs of weevils were caged with each plant and there were 3 replicates of both the test and control. The experiment started on June 22 and the contents of the cages were examined on September 1st. Table II shows the results of this experiment.



Table III. No. of E. villosus oviposition sites in field collected material.

Plants	YST	SF	<u>Carthamus</u> sp.	<u>C. lanatus</u>	<u>C. diffusa</u>
No. Heads	400	400	100	100	100
No.oviposition	72	2 <sup>1/</sup>	-	-	-

1/ It was difficult to decide whether there are oviposition holes or not, because the seed heads were very old and dry. No indication of larvae or larval frass were found in these seed heads.

These several small trials permit us to draw several preliminary conclusions about the suitability of the various plants tested for the feeding, oviposition and development of Eustenopus. The data developed in Test I (Table 1) show the insect has a decided preference for the Greek yellow starthistle.

Test II indicates the insects will feed on safflower to some extent, in the absence of yellowstar but they will not oviposit on safflower.

Test III. This test, indicates that first instar larvae cannot survive on safflower.

Test IV. This test, conducted on field grown plants show that in a replicated trial that the same number of insects make twice as many feeding holes in yellowstar heads as they do in safflower heads, thus demonstrating a marked feeding preference for the weed and complete ovipositional preference.

Test V. In this trial a large number of weevils were confined to safflower. Having 30 heads to choose from, only light feeding and no oviposition was demonstrated.

Test VI. An extensive examination of the susceptible flower heads of several species of plants related to yellow starthistle and occurring in the field near Thermi was made. The only plant with positive oviposition site was yellow starthistle. On safflower two dubious sites were found, since they were on old dried heads they may have been artifacts and not real oviposition sites. No damage was found to the safflower seed.

#### Impact of Eustenopus

The rate of seed destruction by one larva: The seed heads of yellow starthistle from Thermi were divided into 7 categories according to their size from 6-9 millimeters in diameter. In the large heads there were normally a few seeds left but as seen in Table IV no seed was left in the small heads.

Table IV - % of seed consumption by E. villosus in YST seed heads of various sizes.

Diameter of seed heads in mm.	6	6.5	7	7.5	8	8.5	9
No Heads examined	(3)	(4)	(7)	(1)	(10)	(2)	(1)
Average No. of undamaged seeds remaining in infested heads <u>1/</u>	0	0	0.42	0	2	26	1
Average No. of seeds remaining in uninfested heads	20.6	26.7	49.3	45.1	60.1	85.8	89
% seed destroyed	100	100	99.0	100	97.0	70	99

1/ 28 cocoons were found in the infested heads contained 10 adults, 10 living and 8 dead or parasitized pupae.

CENTAUREA DIFFUSA

Diffuse knapweed (Centaurea diffusa Lam) is an herbaceous plant that can be annual or biennial. It was introduced from Europe to the dry grasslands of North America, and was first discovered in the United States in an Alfalfa field in Bingen, Klickitat County, Washington on 1907.

Diffuse knapweed is estimated to infest over 2,000,000 acres in Washington, Oregon and Idaho. The main economic loss from diffuse knapweed is caused by the elimination of superior forage species from the rangeland. As forage knapweed plants have little nutritive value and high fiber content and high levels of consumption can cause toxic symptoms to grazing livestock, especially horses.

Although herbicide treatments can control this toxic weed, chemical treatment is often not cost effective and may have ecologically undesirable side effects.

As an alternative method of abatement, biological control of this weed has been investigated and three insects, to date, have been found to be host specific enough to be released. In 1970, a Tripetidae fly Urophora affinis Frfld, which attacks the seeds of diffuse and spotted knapweed, was released in Canada and later in 1973 in western United States. In the spring of 1976, a root boring beetle, Sphenoptera jugoslavica Obemb., was released in British Columbia (Canada). The third insect is Pterolonche inspersa Stgr. (Lep.: Pterolonchidae). Its biocontrol potential was first noted by Dr. H. Zwolfer, and later serious host specificity testing of this insect was started at the USDA Laboratory, Rome, Italy. The obtained results so far are encouraging.



PTEROLONCHE INSPERSA

R. Sobhian

Thessaloniki, Greece

In order to have large numbers of Pterolonche inspersa eggs to send to the US for release, it was decided to produce them on a C. diffusa garden at Thermi. In mid-March about 1600 rosettes, 2-10 cm in diameter were collected from the field and transplanted into a trial garden. About 10% of the rosettes had died by the end of march so they were replaced with others. It was planned to infest these rosettes with Pterolonche eggs in August but since nearly all of them were flowering, it would not be possible to infest them because they senesce and die soon after flowering. In an attempt to perennialize the plants, thus have them in an acceptable stage for infestation in Augst, some of the plants were pruned, removing the bolting stalk. If this system works, Pterolonche can be reared on field collected plants in large numbers, thus provide an abundant source of females for egg production.

In an attempt to have a garden for 1985, 30 C. diffusa plants were started from seeds in "jiffy sets" in September 1983 and planted in a field at the university farm, in order to find out whether they are going to act like annuals and flower in 1984 or if they will remain rosettes and flower in 1985. The plant is described as a biennial. In March more seeds will be planted for the same purpose.

To provide immature insects to the Rome Laboratory for the study of the biology of Pterolonche inspersa, 200 infested roots of C. diffusa were collected in June near Thermi and mailed to Rome for dissection and study, and two other samples of field collected roots were sent in October and November. The exercise was largely unsuccessful because most of the roots in the last two shipments were not infested.



BANGASTERNUS PROVINCIALIS

Rouhollah Sobhian

B. provincialis is common on diffuse knapweed at Thermi, and is occasionally found on yellow starthistle. There is a heavy mortality during the egg stage. For example, on July 4, 123 eggs were collected in the field and examined under a microscope. Only 7 had hatched normally, the remainder having been parasitized or having dried-up for some unknown reason.

The feeding behavior of the 1st instar B. provincialis larvae on diffuse knapweed is different from that of B. orientalis on yellow starthistle. B. orientalis feeds on the mesophyll tissue of the bract on which the egg is placed, while the provincialis 1st instar larva mines directly into the stem and moves toward the center of the bud.

Also, a single B. provincialis larva can consume the entire contents of a C. diffusa seed head. Since the seed heads are so small, it is doubtful if more than one larva can develop in a single head.

The last instar larvae of B. provincialis make a pupal cell out of the outside row of bracts sticking them together with a hard brownish secretion.

In a sample of C diffusa seed heads collected in July and dissected in November, adult weevils were found resting in pupal cells, inside the head, perhaps to overwinter. B. orientalis adults normally leave the seed heads in September or before.

#### GALL WASP

A tiny gall making wasp attacks C. diffusa in Thermi, ovipositing in the floral buds. The oviposition or the larval feeding turn them into small woody galls. The degree of infestation was very different among individual plants. A sample of these galls was collected on July 5 and they contained eggs, larvae or pupae. Some of the larvae were parasitized so only a few specimens could be reared from the sample. They have not been identified yet.

#### LARINUS MINUTUS

5 specimens believed to be Larinus minutus were also reared from Centaurea diffusa seed heads and sent for confirmation of identification.

PTEROLONCHE INSPERSA

Campobasso, Dunn

In August 1982 experiments (larval survival test) 4 larvae of P. inspersa survived on three species of Centaurea. Two on C. friderici, one on C. cineraria, and one on C. corsiana. In order to see if this moth could complete its development on these three species of Centaurea an experiment was made in August 1983. In addition to several varieties of yellow starthistle an endangered US Cirsium species (Cirsium undulatum) was also included in this test.

Larval survival test: Starting on August 29, 1983 the experiment was set up in the laboratory garden, using potted plants (ambient temperature range 25-30°C, RH 40-80% and photoperiod 14-15 hrs). The following plants were used in the test: Centaurea diffusa (control), C. cineraria, C. friderici, C. corsiana, C. solstitialis var. schowii, and Cirsium undulatum. First instar larvae were transferred with a small brush to the test plants. A total of 5 plants (replications) were made of each test plant using 3 larvae/plant for a total of 15 larvae for each plant species in the test. In order to follow the larval development, 2 replications were dissected in October 20, 1983 finding survival only on the Centaurea diffusa (control); 1 larva on the first replication and 2 larvae on the second one. The remaining 3 replications will be dissected in July 1984.

SPHENOPTERA JUGOSLAVICA

Campobasso, Dunn

During the period from May 14-19 1200 roots of Centaurea diffusa infested with Sphenoptera jugoslavica were collected at Thermi (near Thessaloniki) Greece and sent by air freight to the quarantine at Albany, California.

Even though these roots were infested with mature larvae, and arrived in Albany in good condition, they reported that there was a very small emergence.

ABUTILON THEOPHRASTI

R. Sobhian

Thessaloniki, Greece

Abutilon theophrasti (velvet leaf) seeds germinate very late. By May 4, only small plants, about to 25 cm tall, were found at the University farm Thessaloniki and two different leaf miners were attacking the plants at this early stages. The first one was found feeding only in the cotyledons and the second was found feeding on the mesophyll of true leaves. No adults were reared from these leaf miners.

On May 27 Carcharodus sp.(Lepidoptera) larvae were common, in all stages, feeding on Abutilon leaves. The larvae roll the edges of the leaves and stay mainly in the resulting tube, coming out and to feed on the same leaf. As the larvae grow, they leave the small leaf tubes and make larger ones on other leaves, finally leaving the plants to pupate, probably in the soil or among debris. It seems that the species is multivoltine because larvae in all stages were found feeding on plants throughout the whole season up to the first week of November. From the larvae collected on May 27, the previous year, and kept in a cage outdoors the first adults emerged on June 9. In the period July 15 - August 15, the larvae were less common, but near the end of August, their population increased and on October 13, only a few Carcharodus adults were found resting on Abutilon plants. Many larvae were parasitized or diseased. Normally the insect overwinters as a mature larva and pupates the following spring.

Host Specificity Test:

Ten rolled Abutilon leaves each containing 1 larva, were caged on a potted cotton plant, on May 27 (replicated twice). On May 29, the larvae were

still in the dead Abutilon leaves in the cage and had not moved to the cotton plants. The larvae were then removed from the old Abutilon leaves and put on the cotton leaves. When they were examined on May 30 they had rolled the cotton leaves as they did the Abutilon leaves, and nibbled on the cotton leaves.

Oviposition Test:

The eggs of Carcharodus are brown, nearly spherical, resembling a sea urchin which has lost its spines and are visible to the naked eye, . Three adult moths were caged with a bouquet of Abutilon theophrasti leaves and within two days they had laid several eggs. Later 7 moths were caged for 2 weeks with a potted cotton plant in the same cage used for the Abutilon trial and at the end of that time no eggs were found on the cotton plant.

Field observations:

Also, an unsprayed experimental plot of cotton on the University farm (about 70 m x 70 m) was examined for the presence of Carcharodus on August 30. No eggs were found on any of the cotton plants examined but a small Abutilon plant with 7 leaves was found in the cotton field and it had 10 eggs on it.

A second examination of this cotton field on September 4 again disclosed no Carcharodus on any cotton plant but 3 of the 5 small Abutilon plants found in the field were infested with either eggs or larvae.

A group of 50 Abutilon plants, about 150 meters from the cotton field were examined on August 30 and 45 of them were infested with Carcharodus.

Since a dense population of Abutilon is available at the University farm, where we are located, it is suggested to support a post graduate student to study the phytophagous insects attacking the plant and also study the host specificity of Carcharodus in more detail. Such a study could be done under

our supervision, but the Faculty of Agriculture in Thessaloniki would give the degree to the student.

HELIOTHIS sp.

Unidentified larvae of Heliothis have been found feeding on Abutilon plants throughout the whole season. The larvae were parasitized by at least 2 wasp species which could be of some interest to EPL Paris.

Miscellaneous:

1) A total of 12 samples of diseased plants of Centaurea solstitialis, Centaurea calcitrapa, Centaurea diffusa, Carduus pycnocephalus, Carduus acanthoides, Carduus nutans, Cirsium vulgare, Rumex sp., Cynara scolymus, and Euphorbia seguieriana were collected and sent to Dr. W. Bruckart, USDA, ARS Plant Disease Laboratory, Frederick, Md. with duplicates to Dr. G. Defago, ETH, Zurich.

2) Populations of Centaurea calcitrapa and Cirsium candelabrum were located near Thessaloniki and rosettes of these plants were transplanted to our plot at the University farm. Artichoke root cuttings from the US are also being grown in the plot in preparation for a field test, with B. orientalis in 1984. Also, Carthamus lanatus, Centaurea cyanus, and Centaurea diffusa seeds are being grown for a Bangasternus field test.

Plan for Calendar Year 1984

a) Yellow starthistle: Continue to survey and collect natural enemies in Greece. Emphasis will be placed on equal samples from the same area at regular intervals to determine peak period of activity for each of the associated natural enemies.

b) Yellow starthistle: Conduct a small carefully designed field plot experiment to measure the host specificity preference of the seed head

insects to gather baseline information on competition and interference between species of the seed head niche.

c) Yellow starthistle: A large open field trial using eight plant species and cultivars will be conducted in Greece to determine the host preferences of Bangasternus orientalis and Bangasternus provincialis.

d) Yellow starthistle: Continue studies of the bionomics of the seed head weevil Bangasternus orientalis gathering addition field information on its life history in Greece.

GALIUM Spp.

Clement, Cristofaro

Research objectives for 1983 were to initiate host specificity tests with a flower bud gall-fly (Schizomyia galiorum Kieffer (Dipetra: Cecidomyiidae)), a cecidomyiid stem gall-fly (identity unknown), and a leaf-rolling eriophyid mite. A secondary objective was to conduct a week long survey of northern Italy to locate populations of these arthropods and then to try and colonize them at the Rome Laboratory.

We abandoned plans to conduct definitive tests with the two cecidomyiids, electing instead to spend the available time on trying to determine the worth of a newly discovered leaf-rolling eriophyid mite as a biocontrol agent. This mite was first discovered on April 28 attacking Galium mollugo L. (Rubiaceae) (identity of plant not yet confirmed by a specialist) in a fallow field in the Abruzzo Mountains (elevation about 400 m; located about 140 km from Rome). Galium mollugo in this open field was visably stressed by a high infestation of this mite. This eriophyid may be different (species or biotype) from the one discovered attacking G. mollugo in shaded areas in 1982.

Massimo Cristofaro conducted a survey of northern Italy between June 11 and 18. A brief summary of his survey and the results from all 1983 studies are given below.

Leaf rolling Eriophyid Mite

Specimens of this mite were sent for identification to the Systematic Entomology Laboratory, USDA, in late-August. However, its identity was not established by Dr. E.W. Baker because the taxonomy of European eriophyids is not clear.

On April 28 several infested G. mollugo plants were removed from the collection site in the Abruzzo, placed in clay pots (22 cm diameter) with soil, and transported to the Rome Laboratory where preliminary host specificity tests were run using mites from these potted plants.

#### Test 1

The first test was set up on April 29 on a covered porch along the north side of the laboratory. The test area received direct sun only in late-afternoon.

Instead of clipping off entire leaf-galls from infested plants and attaching them to the stems of healthy and vigorously growing test plants in pots, as we did in the field bindweed-eriphyid mite tests, we formed small clusters of potted plants and draped long, heavily infested stems from a "mite source plant" over the other plants in a cluster. As the galls matured on the source plants the mites migrated to the leaves of the acceptable test plants. In addition to the source plant, there were two other potted plants in a cluster: uninfested G. mollugo (Rome plant) and a test plant. A distance of 20 cm separated clusters. One uninfested potted G. mollugo plant was set off by itself (22 cm from test area) to see if wind blown mites would colonize it.

Additional details and the results are shown in Table 1. The potted G. mollugo plant that was set aside was colonized by the mite in just a few days, indicating the mites can be blown to other plants.

#### Test 2

Another small test was begun on April 30. For this test, however, we clipped off entire leaf-galls on stems and attached them to the test plants. As the leaf-galls dried out we watched to see if the mites migrated to the new plants and successfully colonized them. Each test plant received 8-10 infested leaf whorls of G. mollugo. As in Test 1 we set aside another uninfested G. mollugo plant to serve as a check on mite dispersal.

This test was set up 4 m from Test 1; all of the test plants were in 22 cm diameter pots except for the Mitchellia repens plants, which were in 15 cm diameter pots. A distance of 30 cm separated each of the test plants.

Additional details and the results are shown in Table 2. Once again the "isolated" G. mollugo plant was colonized within a few days after the test was begun.

### Test 3

A third test was set up on June 23 in a shaded area on the grounds of the Rome Laboratory. The methods of Test 1 were used here (ie. cluster of pots; draping branches, etc.).

There were three 4-pot clusters, each comprised of one pot of: G. mollugo (source plant from Abruzzo), uninfested G. mollugo from Rome, G. mollugo from Beltsville, (Maryland, U.S.), and Houstonia caerulea. Another cluster had a pot of Galium sp. (II) in the place of G. mollugo from Beltsville.

No useful information was obtained from this test as the feeding activity of the mite started to decline around late-June. This was substantiated with summer field observations at the Abruzzo site on September 16. However, eriophyids were microscopically observed in leaves of the potted source plants on October 12, which may indicate that the mite becomes active again in the fall.

### Cecidomyid stem-gall fly

Adults of this fly were reared-out from field collected larvae and were sent for identification to the Systematic Entomology Laboratory, USDA, in late-September. This fly was identified as Dasineura sp. by Dr. R.J. Gagne', Systematic Entomology Laboratory, United States Department of Agriculture.

As mentioned above no definitive host specificity tests were

attempted with this fly in 1983 but we did take note of some of its bionomics in the field. One bit of information that may be interesting, however, is that we did observe a stem gall on a potted G. mollugo plant from New York, but adults were not reared out.

We started surveying G. mollugo infestations near the Rome Laboratory during the first week of April and on April 18 the first cecidomyid stem galls were found. Several stems with 100 or more galls were periodically removed from the field between April 18 and 25 and placed in a screened cage with 2 cm of soil in the bottom. As full-grown larvae left these galls and dropped to the soil they were removed and transferred to small plastic cups with 1 cm of soil over a layer of plaster of Paris (1 cm thick). The plaster of Paris helped keep the soil moist. The larvae pupated in the soil and the first adults emerged on May 10.

Heavily galled stems will break and fall over, thus affecting the ability of the plants to produce flowers and seeds. But just as the fly can build-up to high population densities from late-April to late-May, so can the parasitoids which attack the fly. We would be hard pressed to support these statements with good data but our general observations lead us to this tentative conclusion. What data we have relative to field population densities, levels of parasitisms, etc. is outlined below. Samples were collected along Via Vallerano, a road near the Laboratory.

May 4 sample: Twenty-six stems, randomly selected, produced an average of  $7.62 \pm 3.55$  ( $\bar{x} \pm \text{SD}$ ) galls per stem. The number of open galls was  $4.54 \pm 2.37$ ; the number of closed galls was  $3.08 \pm 1.878$ . Forty-seven of the open galls were microscopically examined and were found to be empty. Sixty-one closed galls contained hymenopterous parasitoids. Cecidomyid larvae were found in 12 closed galls.

May 10 sample: Stems from G. mollugo and G. aparine plants growing side-by-side were removed and the number of cecidomyid galls were counted on each stem. No galls were on the 11 stems from G. aparine while 14 stems from G. mollugo supported 27 galls. In some cases, branches from the two species were intertwined.

June 6 sample: Ten plots (90 x 90 cm) were staked out (10 m between plots) and all stems 1.5 mm or larger (in thickness) in these plots were cut and bagged, then returned to the laboratory where the number of cecidomyid flower bud galls (Schizomyia galiorum Kieffer) and stem galls were counted. The results are shown in Table 3.

In anticipation that preliminary host specificity tests might be conducted in 1984 on the grounds of the Rome Laboratory, several G. mollugo and Rubia peregrina plants were dug up on April 15 and transplanted into three plot areas at the Laboratory. The aim is to establish resident populations of cecidomyid gall flies on these G. mollugo so potted test plants can be interspersed among these "fly source" plants. This approach is akin to the method used by P. Pecora in his study of the leafy spurge cecidomyid, Bayeria capitigena (Bremi).

#### Literature Searches

The Review of Applied Entomology, Series A, 1913-73 and the Zoological Record were searched for literature records of Criocoris crassicornis (Hahn)(Hemiptera: Miridae) and Catarhoe rubidata (Denis & Schiffermuller)(Lepidoptera: Geometridae), two insects reported in the 1982 Annual Report as possibly having potential as biocontrol agents. Nothing was found that would indicate that these insects are pests of crop plants; however, those two insects will not receive any attention in 1984.

### Survey of Northern Italy, June 11-18

This survey was conducted in various valleys in the Dolomite range east of Bolzano. In short, Dott. Massimo Cristofaro discovered fairly sizeable infestations of the two cecidomyid flies; however, only a light infestation of eriophyid mites was found. Substantial data were collected on the density of cecidomyid galls (counts were taken on a per plot and per stem basis). The data are not be presented here.

A few black weevils (about 8-10 mm in length) were collected in association with a cluster of G. mollugo plants but their impact on the plant was not apparent. Specimens were identified as Otiorhynchus sp. near pinastri (Herbst) by Dr. D. R. Whitehead, Systematic Entomology Laboratory, United States Department of Agriculture.

Our efforts to start a colony of the Dolomite stem gall-fly at the Rome Laboratory were not successful.

### Concluding Comments

Clement worked up a generalized test plant list for the Galium project in mid-summer, but, like the one developed about 3 or 4 years ago by Dr. S. Batra, it does not include any endangered or threatened species of U.S. Galium. When the Federal Working Group on Biological Weed Control responded in November 1980 to Dr. Batra's list, one member of the group indicated that there are 12 endangered species of Galium in California, Arizona, and Florida and 7 more under consideration for threatened status in California and Texas.

Galium sp. (II) from Maryland (possibly a native species) was severely injured by the mite (as was G. aparine) in the preliminary tests. One member of the Working Group stated in November 1980 that "the problem with G. aparine is that it is a native plant, according to many authors".

The above paragraph points out some potential conflicts and problems that should probably be resolved before any additional studies are undertaken. In any case, Rome personnel will not work on this project in 1984 as it was not one of the four projects given priority status at the November 1983 review of the USDA overseas biological control research programs.

Table 1. Results of a host specificity test (I) with an eriophyid mite, Galium project, Rome, 1983<sup>1/</sup>

Cluster No. and Test Plant <sup>2/</sup>	Estimated No. of stems at Start of Test	Dates of inspection		
		May 9	May 20	June 22
#1				
<u>Galium verum</u> , New York strain, Test plant	200	- <sup>3/</sup>	-	-
<u>Galium mollugo</u> source plant	100 infested stems	+ <sup>4/</sup>	+	+
<u>Galium mollugo</u> Uninfested	50	+	+	+
#2				
<u>Galium aparine</u> Test plant	15	+	+	+
<u>Galium mollugo</u> Source plant	25-30 infested stems	+	+	+
<u>Galium mollugo</u> Uninfested	30	+	+	+
#3				
<u>Galium mollugo</u> Uninfested	30	+	+	+
<u>Galium mollugo</u> Source plant	30-35 infested stems	+	+	+
<u>Rubia peregrina</u> Test plant	7	-	-	-
#4				
<u>Galium mollugo</u> , New York strain, Test plant	100	-	+	+
<u>Galium mollugo</u> Source plant	100 infested stems	+	+	+
<u>Galium mollugo</u> Uninfested	40	+	+	+

<sup>1/</sup> Test set up on 29 April

<sup>2/</sup> All test plants were grown in 22 cm pots.

<sup>3/</sup> (-) = no mites present.

<sup>4/</sup> (+) = mites present and galling leaves.

Table 2. Results of a host specificity test (II) with an eriophyid mite, Galium project, Rome 1983.<sup>1/</sup>

Test Plant	Estimated No. of stems at Start of Test	Date of Infestation		
		May 9	May 20	June 21
<u>Galium aparine</u>	30	+ <sup>3/</sup>	+	Plant dead
<u>Galium mollugo</u> Rome plant	100	- <sup>4/</sup>	+	<u>Eriophyid</u> declined because of 2-spotted spider mite infestation
<u>Rubia peregrina</u> Rome plant	8	-	-	-
<u>Galium verum</u> New York strain	150	-	-	-
<u>Galium mollugo</u> Delemont, Switzerland	40	-	-	-
<u>Galium sp. (II)</u> Native to Maryland	60	+	+	+
<u>Mitchella repens</u> <sup>2/</sup> from Maryland	11	-	-	-

<sup>1/</sup> Test set up on April 30.

<sup>2/</sup> This test plant was grown in 4 pots (15 cm diameter); pots formed a small cluster, whereas the other test plants were grown in one pot.

<sup>3/</sup> (+) = mites present and galling leaves.

<sup>4/</sup> (-) = no mites present.

Table 3. Number of cecidomyid flower bud galls and stem galls from samples of Galium mollugo collected June 6, 1983, along Via Vallerano, Rome.

Plot No.	No. of Stems	No. of Flower Galls per Stem	No. of Stem Galls per Stem	Total No. of Flower Galls	Total No. of Stem Galls
1	4	0,2,0,8	0,0,0,2	10	2
2	4	0,0,0,3	0,2,0,2	3	3
3	15	4,2,1,3,2,0,6,0,10, 8,1,0,5,4,8	0,0,0,0,0,1,0,1, 0,1,0,0,0,1,0	54	4
4	10	0,1,1,0,0,1,1,0,7,0	2,0,3,1,2,2,0,0, 2,0	11	12
5	5	0,0,0,0,3	2,1,2,0,2	3	7
6	14	0,0,1,4,0,0,2,3,2, 0,0,0,9,0	2,1,0,4,1,0,0, 1,1,0,2,3,2,4	21	21
7	8	All 0's	0,4,0,2,0,0,0,1	0	7
8	9	2,0,2,6,0,0,2,0,4	0,0,1,0,0,3,1,1,6	16	12
9	3	1,1,0	0,8,0	2	8
10	8	1,1,0,7,1,2,0,12	0,3,0,0,2,0,0,1	24	6

RUMEX CRISPUS

Clement, Cristofaro

Pyropteron (=Bembecia) chrysidiforme (Esper) (sensu Naumann)

Because only infertile eggs were laid by captive females of this sesiid moth in 1982 we spent a lot of time in 1983 trying to figure out how to obtain fertile eggs so Neal Spencer could conduct more host specificity tests at Stoneville, Mississippi. In Europe we cooperated with Dr. John Scott, CSIRO, Montpellier, France on this problem. Mr. Niklaus Hostettler, a former technician at the Rome Laboratory, also contributed some useful information.

To obtain fertile eggs it is necessary to start with large numbers of adults. To be on the safe side, we think it is important to have at least 75 adults to work with. Next, it is very important to pair freshly emerged males and females (preferably less than one-day old). Mating was repeatedly observed in screened mating cages where the sex ratio was 1-5 males to 10-20 females.

On at least 5 occasions we observed mating within 2 minutes after a freshly emerged male was placed in a cage with several females. A cage was always placed in direct sunlight but as soon as a copulating pair was developed they were removed and placed in a plastic container in a laboratory. Females readily oviposited in these containers. After eggs are laid it is probably important to keep them completely dry to enhance hatching.

We gleaned some useful biological information (moth emergence dates and the extent of tachinid parasitism) during the course of working out the details for getting viable eggs. This information is given in Table 1.

Table 1. Data on adult emergence of Pyropteron chrysidiforme and its habitual tachinid parasitoid. Laboratory study, Rome, Italy 1983.

Insect	Pot No. <u>1/</u>					Cage No. <u>1/</u>	
	1(10) <u>2/</u>	2(6)	3(6)	4(8)	5(6)	1(45)	2(25)
<u>P.chrysidiforme</u>							
No. ♀	1	4	2	3	4	7	11
No. ♂	1	5	1	3	1	5	7
Sex unknown	3	5	6	-	2	2	1
Date of first emergence	June 1	May 17	May 29	June 7	May 26	May 17	June 3
Date of last emergence	June 11	June 27	June 7	June 26	June 17	June 16	June 24
Tachinid fly <u>3/</u>							
No. adults	1	8	4	4	7	15	8
Date of first emergence	June 9	May 19	May 20	June 4	May 20	May 30	June 9
Date of last emergence	-	June 13	June 7	June 14	June 19	June 16	June 16

1/ Roots and lower stems of Rumex crispus were removed from field near Rome on May 6 and returned to the laboratory where they were anchored in clay pots with soil or placed loosely in screened emergence cages. The clay pots were capped with transparent plastic cylinder cages to retain emerging moth and flies. Both pots and cages were held in a temperature-light controlled rearing room at the Rome Laboratory ( $22^{\circ} \pm 2^{\circ}\text{C}$ ; 16 h L; 8 h D). All field collected plant material contained sesiid larvae.

- 2/ Numbers in parenthesis indicate number of infested roots-stems per pot or cage.
- 3/ This fly has been identified as Bithia modesta (Meigen) by Dr. N.E. Woodley, Research Entomologist, Systematic Entomology Laboratory, United States Department of Agriculture, Beltsville, Maryland.

PUBLICATIONS

- Clement, S.L., Rosenthal, S.S., Mimmocchi, T., Cristofaro, M., and Nuzzaci, G. 1983. Concern for U.S. native plants affects biological control of field bindweed. Proc. 10th International Congress of Plant Protection. Vol. 2: 775.
- Buckingham, G.R., Pecora, P. and A. Rizza. 1983. Host specificity test with Stenocarus fuliginosus (Coleoptera: Curculionidae): A Potential Agent for Biocontrol of Illicit Opium Poppy. 12 (1): 24-32.
- Ialongo, M.T., Tedeschi, S., and P. Pecora. 1983. Una popolazione di Puccinia suaveolens (Pers.) Rostr. specifica per il Cirsium arvense (L.) Scop. Annali dell'Istituto Sperimentale per la Patologia Vegetale. Vol. VIII - 1982 - 1983: 81-87.
- Pecora, P., Cianchi, R., Rizza, A., Murano, F., and L. Bullini. 1983. Ricerche genetiche su Chrysolina rossia e Chrysolina gypsophilae: considerazioni tassonomiche ed evolutive (Coleoptera: Chrysomelidae). Atti XIII Congr. Naz. It. Ent.: 75-80.
- Pecora, P., and A. Rizza. 1983. Ricerche sul Controllo Biologico del "complesso" Euphorbia esula-virgata nel Nord America. Atti XII Congr. Naz. It. Ent.: 157-164.

TRAVELS 1983 - ROME & GREECE

January 17 - 18	Clement and Mimmocchi to Bari to confer with entomologists at University of Bari.
February 2 - 4	Dunn, Rizza, Campobasso and Pecora to Piacenza to confer with the Italian Group of Biological Control of Weeds. Cooperative planning meeting USDA, CIBC, CSIRO Laboratories.
February 15 - March 19	Dunn to the U.S. to confer with Headquarters and on Home leave.
March 15 - 16	Clement and Cristofaro to Puglia to survey insects on <u>Centaurea</u> .
March 15 - 17	Rizza and Pecora to Piacenza to collect adults of <u>Oncochila simplex</u> .
April 18 -23	Campobasso to Castel del Monte to collect <u>Centaurea</u> sp.
April 19 - 21	Rizza to Pisa to collect <u>Euphorbia esula</u> plants.
April 25 - June 17	Dunn to Greece and Turkey to survey <u>Centaurea</u> for natural enemies.
April 27 - 28	Clement and Cristofaro to Puglia to collect biocontrol agents on yellow starthistle and related plants.
May 3 - 5	Pecora to Pisa to collect <u>Bayeria capitigena</u> galls.
May 9 - 11	Rizza and Pecora to Pisa to collect galls of the midges <u>Bayeria capitigena</u> and <u>Dasineura capsulae</u> .
May 12 - 23	Campobasso to Greece to collect infested roots of <u>Centaurea diffusa</u> containing <u>Sphenoptera jugoslavica</u> and <u>Pterolonche inspersa</u> . In addition a collection of <u>Bangasternus orientalis</u> was made.
May 26 - 27	Rizza and Pecora to Pisa to collect <u>Oberea</u> adults and galls of <u>Bayeria</u> and <u>Dasineura</u> .
June 7 - July 1	Rizza and Pecora to Austria, and Hungary to collect cold hardy strains of <u>Oberea</u> , <u>Hyles</u> , <u>Bayeria</u> and <u>Dasineura</u> on <u>E. virgata</u> .
June 13 - 18	Cristofaro to Bolzano to survey insects on <u>Galium</u> .
June 13 - 19	Stazi to Pisa to collect <u>Aphthona flava</u> , <u>Hyles euphorbiae</u> , <u>Oberea</u> sp. <u>Dasineura</u> and <u>Bayeria</u> galls.

June 27 -29	Clement and Cristofaro to Puglia to collect <u>Apion</u> and other insects on yellow starthistle.
July 15 - 16	Dunn and Clement to Puglia to collect <u>Urophora</u> flies on yellow starthistle.
July 27 - 29	Pecora and Stazi to Pisa to collect <u>Dasineura capsulae</u> galls.
August 3 - 4	Stazi to Pisa to collect <u>Dicranocephalus</u> sp., <u>Bayeria</u> galls and <u>Euphorbia esula</u> plants.
August 17 - 18	Stazi to Pisa to collect <u>Dicranocephalus</u> sp. and <u>Euphorbia esula</u> plants.
Aug. 31 - Sept 1	Pecora and Stazi to Pisa to collect <u>Aphthona abdominalis</u> .
Sept. 27 - 29	Clement and Cristofaro to Puglia to collect seed head insects on yellow starthistle.
Sept. 27 - Oct. 6	Pecora and Stazi to France to collect <u>Chrysolina rossia</u> and <u>C. gypsophilae</u> .
Oct. 6 - 12	Rizza to Calabria to collect roots of <u>Carduus nutans</u> infested by mature larvae of <u>Cheilosia corydon</u> .
Oct. 11 - 13	Dunn and Pecora to Piacenza to attend the annual meeting organized by the Italian Group of Biological Control of Weeds.
Oct. 16 - 20	Dunn to Delemont to attend meeting with CIBC, CSIRO to develop cooperative support.
Oct. 18 - 24	Rizza and Pecora to Austria to collect galls of <u>Urophora cardui</u> .
Nov. 7 - 19	Sobhian to Italy and Paris to confer with peers and participate to the Biological Control of Weeds Laboratory Review.
Nov. 13 - 18	Dunn, Campobasso, Pecora, Clement, Magni to Paris to attend the Biological Control of Weeds Laboratory Review.
Nov. 19 - 24	Dunn and Magni in Paris to participate to the Administrative Review.
Nov. 19 - Jan 5	Clement to Brighton, England to attend a Meeting and to work at the British Museum in London, to confer with USDA scientists at Beltsville, and to take Home Leave.

Nov. 25 - Dec. 16

Magni to the U.S. to attend a Seminar in Washington, visit the NFC in New Orleans, visit the Biological Control of Weeds Laboratory in Albany.

Dec. 12 - 13

Pecora to Naples and Bari to visit Prof. E. Tremblay and Prof. M. Solinas respectively.

- 111 -  
INSECT SHIPMENTS FROM ROME LAB.

Host Weed(s)	Location	No. Stage Date	Shipping Method	Receiving Location
ABUTILON THEOPHRASTI Pathogens	Lombardia (Italy)	6 leaves 7/8/83	APO	Frederick, MD.
CENTAUREA DIFFUSA <u>Sphenoptera</u> <u>jugoslavica</u>	Thermi, (Greece)	200 l., p., a., 1/6/83	Airfreight	Albany, CA.
<u>Sphenoptera</u> <u>jugoslavica</u>	Thermi, (Greece)	1000 l., p., a.	Airfreight	Albany, CA.
CENTAUREA SOLSTITIALIS <u>Urophora siruna-seva</u>	Puglia (Italy)	9181 floral buds 5/21/83	Airfreight	Albany, CA.
CIRSIIUM ARVENSE <u>Urophora cardui</u>	Vienna (Austria)	4000 galls 4/12/83	Airfreight	Albany, CA.
EUPHORBIA ESULA <u>Aphthona flava</u>	Pisa (Italy)	300 adults 6/21/83	Airfreight	Albany, CA.
<u>Aphthona flava</u>	Pisa (Italy)	1260 adults 6/21/83	Airfreight	Regina Saskatchewan Canada
<u>Aphthona flava</u>	Hungary	190 adults 6/27/83	Airfreight	Albany, CA.
<u>Aphthona cyparissias</u>	St. Polten (Austria)	103 adults 7/8/83	Airfreight	Albany, CA.
<u>Chamaesphecia</u> <u>empiformis</u>	Tocaj (Hungary)	500 roots 4/19/84	Airfreight	Albany, CA.
<u>Oberea erythrocephala</u>	Pisa (Italy)	100 adults 6/21/83	Airfreight	Zurich, CH.
<u>Oberea erythrocephala</u>	Pisa (Italy)	142 adults 7/6/83	Airfreight	Albany, CA.
EUPHORBIA rusts	Pisa	4/27/83	Airfreight	Frederick, MD.
EUPHORBIA VIRGATA <u>Hyles euphorbiae</u>	Debrecen (Hungary)	1500 eggs 7/26/83	Airfreight	Albany, CA.
<u>Hyles euphorbiae</u>	Debrecen (Hungary)	85 pupae 8/30/83	Airfreight	Albany, CA.
RUMEX CRISPUS <u>Pyropterion</u> <u>chrysidiforme</u>	Rome (Italy)	60 larvae 1/12/83	Airfreight	Stoneville, MS.
<u>Pyropterion</u> <u>chrysidiforme</u>	Rome (Italy)	578 eggs 6/8/83	Airfreight	Stoneville, MS.

l. = larvae; a. = adults; p. = pupae.

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Several colleagues from the University of Thessaloniki and from the Plant Protection Institute, Thessaloniki.

40 students of the Faculty of Agriculture, Thessaloniki visited our laboratory and our experimental plot at the University farm. They were divided in two groups. The purpose of their visit was to learn the principles of biological control of weeds, the methods we use for testing the promising candidates and the purpose of our survey in Greece.

#### ACKNOWLEDGMENTS - GREECE

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